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Immunotherapy

A Phase 1 Trial of CNDO-109–Activated Natural Killer Cells in Patients with High-Risk Acute Myeloid Leukemia

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Todd A. Fehniger [1],*, Jeffrey S. Miller [2], Robert K. Stuart [3], Sarah Cooley [2], Amandeep Salhotra [4], Julie Curtsinger [2], Peter Westervelt [1], John F. DiPersio [1], Timothy M. Hillman [5], Nova Silver [5], Michael Szarek [6], Leonid Gorelik [5], Mark W. Lowdell [7], Eric Rowinsky [5]

[1] Division of Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri
[2] Department of Medicine, University of Minnesota, Minneapolis, Minnesota
[3] Department of Hematology and Oncology, Medical University of South Carolina, Charleston, South Carolina
[4] Department of Hematology and Hematopoietic Cell Transplantation, Beckman Research Institute of City of Hope, City of Hope Comprehensive Cancer Center, Duarte, California
[5] Fortress Biotech, Inc., New York, New York
[6] Department of Epidemiology and Biostatistics, SUNY Downstate School of Public Health, Brooklyn New York
[7] Department of Hematology, Royal Free Hospital, UCL Medical School, London, United Kingdom

A B S T R A C T

Natural killer (NK) cells are an emerging immunotherapy approach to acute myeloid leukemia (AML); however, the optimal approach to activate NK cells before adoptive transfer remains unclear. Human NK cells that are primed with the CTV-1 leukemia cell line lysate CNDO-109 exhibit enhanced cytotoxicity against NK cell–resistant cell lines. To translate this finding to the clinic, CNDO-109–activated NK cells (CNDO-109-NK cells) isolated from related HLA-haploidentical donors were evaluated in a phase 1 dose-escalation trial at doses of 3×10^5 (n = 3), 1×10^6 (n = 3), and 3×10^6 (n = 6) cells/kg in patients with AML in first complete remission (CR1) at high risk for recurrence. Before CNDO-109-NK cell administration, patients were treated with lymphodepleting fludarabine/cyclophosphamide. CNDO-109-NK cells were well tolerated, and no dose-limiting toxicities were observed at the highest tested dose. The median relapse-free survival (RFS) by dose level was 105 (3×10^5), 156 (1×10^6), and 337 (3×10^6) days. Two patients remained relapse-free in post-trial follow-up, with RFS durations exceeding 42.5 months. Donor NK cell microchimerism was detected on day 7 in 10 of 12 patients, with 3 patients having evidence of donor cells on day 14 or later. This trial establishes that CNDO-109-NK cells generated from related HLA haploidentical donors, cryopreserved, and then safely administered to AML patients with transient persistence without exogenous cytokine support. Three durable complete remissions of 32.6 to 47.6+ months were observed, suggesting additional clinical investigation of CNDO-109-NK cells for patients with myeloid malignancies, alone or in combination with additional immunotherapy strategies, is warranted.

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INTRODUCTION

Acute myeloid leukemia (AML) is a clinically challenging myeloid malignancy predominantly affecting older patients with a 5-year survival rate of 26% [1,2]. Importantly, AML patients who are older than 60 years or have other high-risk factors have lower survival rates that have not changed substantially over the past 3 decades [3]. Although initial chemotherapy approaches may result in a complete remission (CR) in a large proportion of older AML patients, these responses are not durable, and consolidation therapy is necessary for long-term disease-free survival [1]. Hematopoietic cell transplantation (HCT) is an effective therapy for patients via antileukemia immune responses mediated by alloreactive T and natural killer (NK) cell responses [1,3]. However, most older AML patients are not candidates for HCT because of treatment-related morbidity and mortality. New adoptive cellular therapy strategies that harness the antileukemia immune properties of NK or T cells are a promising approach to provide cellular immunotherapy for AML without HCT.

NK cells are an important component of innate immunity and mediate antileukemia responses [4,5]. Unlike adaptive

* Correspondence and reprint requests: Todd A. Fehniger, MD, Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, MO.
E-mail address: tfehnige@wustl.edu (T.A. Fehniger).

lymphocytes that recognize target cells via clonal antigen-specific activating receptors, NK cells integrate signals from a broad array of inhibitory and activating receptors, facilitating rapid response to tumor target cells [6]. Naive NK cells require multiple signals to potently respond robustly to target cells [7]. Such requirements can be induced by a "priming" signal that activates the NK cell without degranulation and a "triggering" signal mediated via 1 of several activating receptors interacting with ligands present on the tumor cell. However, many tumor cells do not prime an NK cell for a potent triggering response, resulting in an NK cell–resistant tumor. To effectively respond to resistant tumor cells, naive NK cells require 1 of several "priming" signals mediated by cytokine receptors (eg, IL-2, IL-15, IL-12, IL-21) [8] or cell-surface priming receptors [9]. Several clinical studies have used IL-2–activated [10-13] or IL-12/-15/-18–activated [14] HLA-haploidentical NK cells, demonstrating the feasibility and safety of this NK immunotherapy approach.

The CNDO-109–activated allogeneic NK cells (CNDO-109-NK cells) were developed based on the scientific rationale of providing a short-term priming signal ex vivo, not via cytokine receptors, but instead via the CTV-1 leukemia cell lysate and NK cell receptor interactions [15]. Such leukemia-primed NK cells are reactive to a wide range of hematologic malignancies and exhibit cytotoxicity against NK cell-resistant targets [9,16]. CNDO-109-NK cells consist of HLA-haploidentical NK cells isolated from a related donor, followed by activation with a current Good Manufacturing Practices compliant lysate derived from the CTV-1 tumor cell line (CNDO-109), and viable cryopreservation. In a small pilot trial CTV-1–activated allogeneic NK cells were administered to 7 patients with AML in CR, partial remission, or in second CR (CR2) after conditioning with fludarabine and total body irradiation [16]. The treatment was well tolerated with expected cytopenias; infusion reactions and graft-versus-host disease (GVHD) were not observed. One patient in first partial remission converted to a CR that was durable after this NK cell therapy. Based on these promising findings, CNDO-109-NK cells were investigated in a phase 1 trial of high-risk AML patients in CR1 at the time of treatment, prepared for adoptive NK cells with a more standard fludarabine/cyclophosphamide immunosuppressive conditioning.

METHODS
Trial Design
This was a phase 1, nonrandomized, open-label, dose-escalation multicenter trial of CNDO-109-NK cells in adults with AML in CR1 who were considered at high risk for recurrence (NCT01520558). The institutional review boards at all participating sites approved the trial, and all patients provided informed consent before the performance of any trial-related procedures. The trial used a traditional 3 + 3 dose escalation design, with the primary objective of defining the maximum tolerated dose or maximum tested dose at which dose-limiting toxicities (DLTs) were not observed. Evaluation of the overall safety profile and duration of relapse-free survival (RFS) and overall survival (OS) were secondary objectives. Chimerism and persistence of donor NK cells were evaluated on an exploratory basis. DLT was defined as the occurrence of absolute neutrophil counts < 500/µL 28 days after infusion of trial product not due to recurrence of AML, trial product-related grade ≥ 3 allergic reaction, or any trial product-related grade ≥ 4 organ toxicity within the first 30 days after trial product administration. Patients were treated in escalating doses of trial product at doses of 3×10^5, 1×10^6, or 3×10^6 cells/kg recipient body weight after preparative chemotherapy. The maximum tolerated dose was defined as the highest dose level at which ≤1 of 6 patients experienced a DLT.

Eligibility
Adult patients aged ≥ 18 years with AML who had achieved CR1 in the previous 16 weeks and were not considered candidates for HCT and at high risk for recurrence were eligible. High risk for recurrence was defined as the

presence of ≥1 of the following: high-risk cytogenetics (-5, -7, del[5q], abnormal 3q, 11q23 translocations, complex cytogenetics) or if cytogenetics were normal, the presence of an FLT3 mutation without an NPM1 mutation; age ≥ 60 years; an antecedent hematologic disorder; and therapy-related AML. Patients were required to have at least 1 eligible HLA-haploidentical donor to produce the trial product. If multiple eligible donors were available, the treating physician chose the most suitable donor based on age, serologic test results, and venous access; KIR to KIR ligand mismatch was not used to select donors. Patients who had received a prior HCT were not eligible.

Generation of CNDO-109-NK Cell Products
CNDO-109-NK cells were prepared from a single leukapheresis product of peripheral blood (PB) mononuclear cells from a healthy, HLA-haploidentical, first- or second-degree relative. Leukaphereses were shipped from the donor apheresis site to a central processing facility. NK (CD56+) cells were purified from PB mononuclear cells with a CD56 MultiSort kit by CliniMACS (Miltenyi Biotech, Auburn, CA). NK cells were incubated ex vivo with CNDO-109 Lysate for 16 hours before lysate removal and cryopreservation of the activated NK cell product. Cytokines were not used in the incubation process. The incubation of NK cells with CNDO-109 lysate resulted in activated NK in all cases. The release criteria for activated NK products included sterility, undetectable bacterial endotoxin, absence of mycoplasma, viability > 70%, and potency, as determined by increased potential to lyse NK-resistant tumor cells compared with the nonactivated NK cells from the same donor. The final product was released after quality control testing as a single, cryopreserved dose of CNDO-109-NK cells from the patient-specific donor and was shipped to the clinical site before chemotherapy conditioning.

Patient Treatment
All patients in the first (3×10^5/kg) and second (1×10^6/kg) dose levels received the planned doses of CNDO-109-NK cells/kg patient body weight. Six patients were entered into the final cohort, of whom 5 received the target dose of 3×10^6 CNDO-109-NK/kg and 1 received 2.5×10^6 because of an inadequate yield of donor NK cells. For safety analyses this patient was included in the 3×10^6 group. Excepting this latter patient, all products met release criteria (median CD56+CD3− purity, 71.8% [range, 55.5% to 93.8%]; median viability, 96.4% [range, 92.5% to 99.2%]).

Before the infusion of CNDO-109-NK cells, each patient was conditioned with cyclophosphamide 60 mg/kg on day –5 and fludarabine 25 mg/m²/day (20 mg/m²/day for patients with creatinine clearance ≤ 70 mL/min) based on actual body weight on days –6 to –2. CNDO-109-NK cells were thawed at bedside and administered without washing on day 0 via a single slow push i.v. infusion over approximately 10 minutes. After CNDO-109-NK cell infusion, patients were followed weekly through month 1, then biweekly through month 3, and then monthly through month 12 post-treatment. Thereafter, patients who remained on-trial were followed on an every-3-month basis for survival and disease status in a survival extension period. The adverse event (AE) reporting period began with the initiation of the preinfusion preparative therapy and continued through 30 days postinfusion. Patients were enrolled from 2013 ro 2014.

Correlative Studies
PB was sampled pretreatment and postinfusion on days 7, 14, 28, 56, 112, and 170, and bone marrow (BM) samples were collected on days 56, 112, and 170. Samples were assessed at a central laboratory (UMN Translational Cell Therapy, Minneapolis, MN) for molecular and flow cytometry chimerism and NK cell characterization. The presence and persistence of donor NK cells was determined using molecular (DNA) chimerism analysis with short tandem repeat genotyping, a standard technique used to monitor engraftment after allogeneic HCT. To increase the likelihood of detecting small numbers of infused cells, a selection process was performed using an NK magnetic column selection kit (Miltenyi Biotech). When feasible, donor-specific HLA mAbs and flow cytometry were used to track donor NK cells in vivo after adoptive transfer.

For all patients, flow cytometry analysis was done to determine the frequency of NK cells (CD3−CD56+ lymphocytes) in blood and BM samples before and at multiple times after product infusion. For 8 of 12 patients additional characterization of NK cells by flow cytometry was done by evaluating expression of CD69, CD16, NKG2A, and CD57 on NK cells.

Response Assessment and Statistics
Leukemia assessments were performed within 14 days before the start of preinfusion preparative therapy and every 2 months thereafter through 12 months of follow-up. Response and progression data were reported based on the International Working Group (IWG) response criteria for AML [17]. Time to event endpoints included duration of RFS and OS through 12 months of follow-up. All patients who received CNDO-109-NK cells were included in analyses of safety and leukemia status. AEs were summarized using the

Common Terminology Criteria for Adverse Events, v4.03. Acute infusion-related toxicity within 6 hours after infusion was summarized separately. Cytopenias and GVHD were summarized as safety data of interest.

RFS duration was prospectively defined as the interval from the date of trial product infusion to the date of relapse (according to IWG criteria for AML) or death as well as from CR1 to the date of relapse. Patients who died without documented relapse were censored on the date of death. Patients who did not progress through month 12 or were lost to follow-up before month 12 were censored at the day of their last leukemia assessment. If no postbaseline leukemia assessment was available, the patient was censored at the date of infusion. If death or relapse occurred after 2 or more consecutive missing leukemia assessments, censoring occurred at the date of the last assessment. The use of a new antileukemic therapy before the occurrence of relapse resulted in censoring at the date of last assessment before the initiation of new therapy. OS duration also was analyzed using the prospective definition of from the date of trial product infusion as well as from the date of CR1 to the date of death. If the patient was alive at the end of the follow-up period or was lost to follow-up, OS duration was censored on the last date the patient was last known to be alive. Distributions of RFS and OS were summarized by dosing cohort. Efficacy data were analyzed using the Full Analysis Set, defined identically to the Safety Population, and also using the per-protocol population, defined as all patients in the Full Analysis Set who had at least 1 efficacy assessment and were without any major protocol violations.

RESULTS

Patient Characteristics and Trial Treatment

Twelve patients were enrolled and treated with CNDO-109-NK cells. Baseline characteristics of each patient are listed in Table 1. The median age was 73 years (range, 57 to 79). At diagnosis, 7 patients (58%) had de novo AML and 5 (42%) had secondary AML. All patients were considered to have high-risk disease, based on age or cytogenetic profile (Table 1). Median time since diagnosis was 107 days (range, 81 to 180). All patients had received induction therapy, per protocol, with 11 patients (92%) having achieved CR1 within 10 weeks of screening.

All 12 patients were evaluated for safety. Ten of 12 patients were included in the per-protocol population, with 2 patients excluded because of protocol violations. One patient (3×10^6 group) with a history of large B cell lymphoma and Burkitt lymphoma who subsequently developed therapy-related myelodysplastic syndrome had not achieved CR1 before screening; a waiver for enrollment was granted. The second patient (3×10^5 group) was excluded because of informed consent violations; 2 donors for this patient were HLA typed before provision of written informed consent. A trial product ultimately was prepared for this patient using PB mononuclear cells from a third consented donor.

Safety and DLTs

No DLTs were reported; therefore, the highest dose of CNDO-109-NK cells evaluated, 3×10^6 cells/kg patient body weight, was identified as the maximum tested dose. Further dose-escalation was not planned, as 3×10^6 cells/kg represented the technical limit of production. CNDO-109-NK cells were well tolerated; no infusion-related events were reported, and no patient developed GVHD. An overall summary of treatment-emergent AEs (TEAEs) is presented in Table 2. All 12 patients experienced TEAEs, most commonly fatigue (50%) and febrile neutropenia, neutropenia, and thrombocytopenia (each 42%) (Table 3). Eight patients (67%) experienced grade 3 or 4 TEAEs, most commonly expected hematologic abnormalities, including febrile neutropenia and neutropenia (each 33%), thrombocytopenia and WBC count decrease (each 25%), and anemia (8%). Nonhematologic grade 3 TEAEs, each reported in 1 patients (8%), included dyspnea, oliguria, and septic shock, all unrelated to trial product. No grade 4 nonhematologic TEAEs were reported.

As expected with the immunosuppressive fludarabine/cyclophosphamide chemotherapy, 8 patients (67%) experienced at least 1 "neutropenic event," including febrile neutropenia, neutropenia, and/or decreased neutrophil count. The time to onset was variable, ranging from 0 to 27 days post-treatment (median, 8 days). All such events were grade 3 or 4 in intensity, with the exception of 1 case of grade 1 febrile neutropenia. Neutropenic events are an expected result of the conditioning regimen and accordingly were considered unrelated to CNDO-109-NK cells in all but 1 case. A neutropenic event was serious for 4 patients. Two patients required growth factor support (granulocyte colony-stimulating factor).

One patient in the 3×10^6 group, a 66-year-old white man with therapy-related myelodysplastic syndrome and a history of atrial fibrillation who had been granted a waiver for enrollment, died due to atrial fibrillation with rapid ventricular response in the setting of disease progression 8 weeks post-treatment. This event was assessed as unrelated to both the trial product and conditioning regimen. Overall, 5 patients (42%) experienced serious AEs. In addition to atrial fibrillation, serious AEs included febrile neutropenia (33%) and single incidences (each 8%) of neutropenia and creatinine increase (1.8 mg/dL; normal range, .7 to 1.2) in the setting of dehydration. All serious AEs were considered by the investigators to be unrelated to CNDO-109-NK cells.

Relapse-Free Survival

The median RFS for all patients and per-protocol patients, defined from the date of CR1, are shown in Figure 1 and Table 4. The RFS rate at 12 months was 33% in each dose level for all patients. The RFS rates at 12 months were 50% (dose level 1), 33% (dose level 2), and 40% (dose level 3). Results for RFS when defined from the date of trial product infusion also are presented in Table 4. Of note, based on post-trial follow-up, 3 patients had long-term RFS of 32.6, 42.5+, and 47.6+ months, with 2 of these 3 patients remaining relapse-free. The duration of CR1 before trial product infusion ranged from 58 to 139 days among these 3 patients. Note that 1 patient in the 3×10^6 group with a duration of CR1 of 32.6 months was treated with sorafenib 200 mg as maintenance therapy starting on day 93 after NK cell treatment and continuing for approximately 2+ years; per protocol, such treatment was permissible and may have contributed to the prolonged CR.

Donor NK Cell Chimerism and Persistence

As determined using 1 or both chimerism testing methods (molecular DNA chimerism with short tandem repeat genotyping after CD56-positive selection and flow cytometry), 11 of 12 patients had evidence of circulating donor cells. Molecular chimerism analysis of the NK-enriched mononuclear cell population from day 7 blood detected donor DNA in 9 patients, and 1 patient had detectable donor DNA on day 14 but not on day 7. For 5 patients, chimerism testing returned results of only 1% or 2% donor DNA, below the assay quantitative limit. For the remaining 5 patients, detectable donor DNA ranged from 11% to 84% (Table 5). Three patients had detectable donor DNA on day 14 or later.

Six of 12 patients had a recipient–donor HLA allele difference that allowed for tracking by flow cytometry. By flow cytometry chimerism analysis, 5 of 6 patients, including 2 with molecular chimerism results of <2% donor DNA in the NK-enriched population, had donor cells detectable in the blood on day 7, with frequencies ranging from .4% to 66.2% of NK cells expressing the donor HLA allele (Figure 2A). The sixth

Table 1
Demographics, Baseline Disease Characteristics, and Outcomes by Patient

Patient	Age (yr)	Sex	Baseline ECOG PS	De Novo vs Secondary AML	Cytogenetic Risk and Age Category	KIR m/m GVL[*]	Induction Therapy	No. of Cycles	Duration of CR1 at Treatment	Donor NK Cells Day 7[†] (%)	RFS (days)[‡]	OS (days)[§]
3 × 10^5												
3-001	74	F	0	De novo	Adverse Del(5q)	No	Cytarabine; daunorubicin liposome (CPX-351)	1	65	0	92	98
3-002	72	F	0	De novo	Adverse Del (7)	Bw4 C2	Cytarabine, daunorubicin	1	71	2	525	525
8-002	73	F	1	Secondary	Adverse Del (5)	Bw4	Cytarabine, Daunorubicin	1	49	0	105	410
1 × 10^6												
2-001	79	M	0	De novo	Unknown age >60	C2	Clofarabine, cytarabine	1	58	2	1448+ [‖]	1448+ [‖]
3-004	74	M	0	Secondary	Unknown age >60	No			88	15	117	131
3-005	76	F	1	Secondary	Unknown age >60	C2	Clofarabine	1	33	1	156	232
3 × 10^6												
2-002	75	M	0	De novo	Unknown age >60	C2	Cytarabine, idarubicin	1	28	15	344	347
3-006	67	M	0	Secondary	Unknown Therapy-related AML	No	Cytarabine, idarubicin	1	139	2	1292 + [‖]	1292 + [‖]
5-001	57	M	1	De novo	Intermediate 2 FLT3-ITD mutation w/o NPM1 mutation	No	Cytarabine, daunorubicin, idarubicin	1	117	0	991[‖]	991[‖]
8-004	73	M	0	De novo	Unknown age >60	Bw4 C2	Cytarabine, daunorubicin	1	89	84	183	241
8-005	66	M	1	Secondary	8% blasts; myelodysplastic syndrome phenotype; 5.9% MRD, age > 60	Bw4	—	—	—	11	176	176
8-006	66	M	2	De novo	Age > 60	No	Cytarabine, daunorubicin	1	167	64	330	336

Cytogenetic risk category was defined as described [18]. ECOG indicates Eastern Cooperative Oncology Group; PS, Performance status; GVL, graft-versus-leukemia.

[*] Donor and recipient HLA were used to identify KIR ligand mismatch in the GVL direction. The mismatched allele or if there was no mismatch is indicated.

[†] Donor NK cells (%), as detected by molecular DNA chimerism with short tandem repeat genotyping.

[‡] RFS from CR1 was defined as the time from the date of CR1 until the date of relapse or death due to any cause. For RFS from CR1, patients who died without documented relapse were considered to have relapsed on the day of their death. If patients did not progress through 12 months of follow-up or were lost to follow-up before the 12-month follow-up visit, they were censored at the day of last disease status assessment.

[§] OS from CR1 was defined as the time from the date of CR1 until the date of death from any cause. For OS durations, if the patient was alive at the end of the follow-up period or was lost to follow-up, OS duration from CR1 was censored on the last date the patient was known to be alive.

[‖] Based on post-trial follow-up.

Table 2
Overall Summary of TEAEs, Overall and by Dose Group

Patients with ≥1	Dose Group			Total (n = 12)
	3×10^5 (n = 3)	1×10^6 (n = 3)	3×10^6 (n = 6)	
TEAEs	3 (100)	3 (100)	6 (100)	12 (100)
Study drug-related TEAEs	3 (100)	2 (67)	4 (67)	9 (75)
DLTs	0	0	0	0
TEAEs representing an infusion-related toxicity	0	0	0	0
Grade 3 TEAEs	1 (33)	1 (33)	1 (17)	3 (25)
Grade 4 TEAEs	1 (33)	1 (33)	3 (50)	5 (42)
Grade 5 TEAEs (ie, TEAEs resulting in death)	0	0	1 (17)	1 (8)
Serious AEs	1 (33)	1 (33)	3 (50)	5 (42)
TEAEs leading to study discontinuation	0	0	1 (17)	1 (8)

Values are n (%).

patient did not have sufficient cells to evaluate by flow chimerism on day 7; molecular chimerism showed 84% donor DNA in the NK cell–enriched population. This patient also had a small but detectable population of donor-activated NK cells in blood on days 14, 28, and 56 and in BM on day 56 (Figure 2A-D).

Phenotypic analysis of NK cells was performed for 8 patients; the parameters examined were CD69, CD16, NKG2A, and CD57. Of the 8 patients evaluated, 4 had an increase from baseline in CD69 expression on NK cells at day 7, which was sustained through day 28 or 56 (Figure 2).

Because few, if any, donor NK cells were detected after day 7 in any patient, these results suggest that the infused NK cells or treatment regimen may have activated endogenous NK cells [19]. No consistent pattern of change in expression relative to baseline of the other NK markers examined was observed (data not shown).

DISCUSSION

A new strategy to enhance NK cell antileukemia activity via priming with a tumor lysate was tested in a multicenter phase 1 clinical trial. AML patients in CR1 underwent consolidation therapy with lymphodepleting chemotherapy followed by a single dose of CNDO-109-NK cells. The experimental CNDO-109-NK product was successfully generated for all patients at a central cellular processing facility, with all NK products meeting lot release criteria. This cell therapy–based consolidation treatment was well tolerated, with expected conditioning related cytopenias, and the maximum tested dose was defined as 3×10^6 CNDO-109-NK cells/kg. Donor NK cells were detectable in most patients at day 7 postinfusion. Of the 12 high-risk AML patients treated, 3 had remarkably durable CRs of 33 to 48+ months after therapy, with 2 remaining relapse free.

Thus, this trial demonstrates the feasibility and safety of generating CNDO-109-NK cells and administered to patients with AML and supports continued investigation in phase 2 clinical trials in patient with myeloid diseases. Several notable strengths of CNDO-109-NK cells include temporal flexibility of administration, limited ex vivo stimulation, and the potential for multiple doses. The CNDO-109 primed donor NK cell products were viably cryopreserved and exhibited increased activity against NK resistant targets compared with unprimed cells, which was evident after cell thaw. The ability to cryopreserve provides greater flexibility than many comparable cellular products and the potential for multidose or retreatment options. Because donor NK cells are primed overnight and then infused into the patient, potential drawbacks of "expansion addiction" that occur after prolonged culture and large-scale expansion with NK cellular products are avoided. Based on these favorable properties, CND0-109-NK cells may have a unique place among published NK cell therapy methodologies.

This study was not designed to characterize the CND0-109-NK product before infusion. This limitation will be addressed by a comprehensive analysis of activating and inhibitory receptor expression on CNDO-109-NK in a planned phase 2 study.

As a trial designed to investigate CNDO-109-NK cells for AML patients in CR, a direct assessment of remission induction was not intended. However, prolonged CRs in this high-risk group of AML patients were notable. Two patients, 1 aged 78 years (1×10^6 group) and another aged 57 years (3×10^6 group), had a diagnosis of de novo AML, with the latter having cytogenetic evidence of a FLT3-ITD mutation without an NPM1 mutation. The third patient (3×10^6 group) was a 67-year-old with an inv(16) AML arising in the setting of myelodysplastic syndrome after treatment for follicular lymphoma. CRs of this duration are unexpected in high-risk AML patients not otherwise consolidated, particularly elderly patients and those with an FLT3-ITD mutation [20]. These clinical results are suggestive of antileukemia activity but require

Table 3
Most Common (Incidence ≥ 25%) TEAEs Overall and by Dose Group

MedDRA preferred term	Dose Group						Total (n = 12)	
	3×10^5 (n = 3)		1×10^6 (n = 3)		3×10^6 (n = 6)			
	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Patients with ≥1 TEAE	3 (100)	2 (67)	3 (100)	2 (67)	6 (100)	4 (67)	12 (100)	8 (67)
Hematologic TEAEs								
Febrile neutropenia	2 (67)	2 (67)	1 (33)	1 (33)	2 (33)	1 (17)	5 (42)	4 (33)
Neutropenia/neutrophil count decreased	0	0	1 (33)	1 (33)	4 (67)	3 (50)	5 (42)	4 (33)
Thrombocytopenia/platelet count decreased	1 (33)	1 (33)	1 (33)	0	3 (50)	2 (33)	5 (42)	3 (25)
WBC count decreased	0	0	1 (33)	1 (33)	2 (33)	3 (33)	3 (25)	3 (25)
Nonhematologic TEAEs								
Fatigue	2 (67)	0	1 (33)	0	3 (50)	0	6 (50)	0
Anxiety	1 (33)	0	0	0	3 (50)	0	4 (33)	0
Diarrhea	0	0	2 (67)	0	2 (33)	0	4 (33)	0
Hypoalbuminemia	0	0	1 (33)	0	2 (33)	0	3 (25)	0
Hypotension	1 (33)	0	0	0	2 (33)	0	3 (25)	0

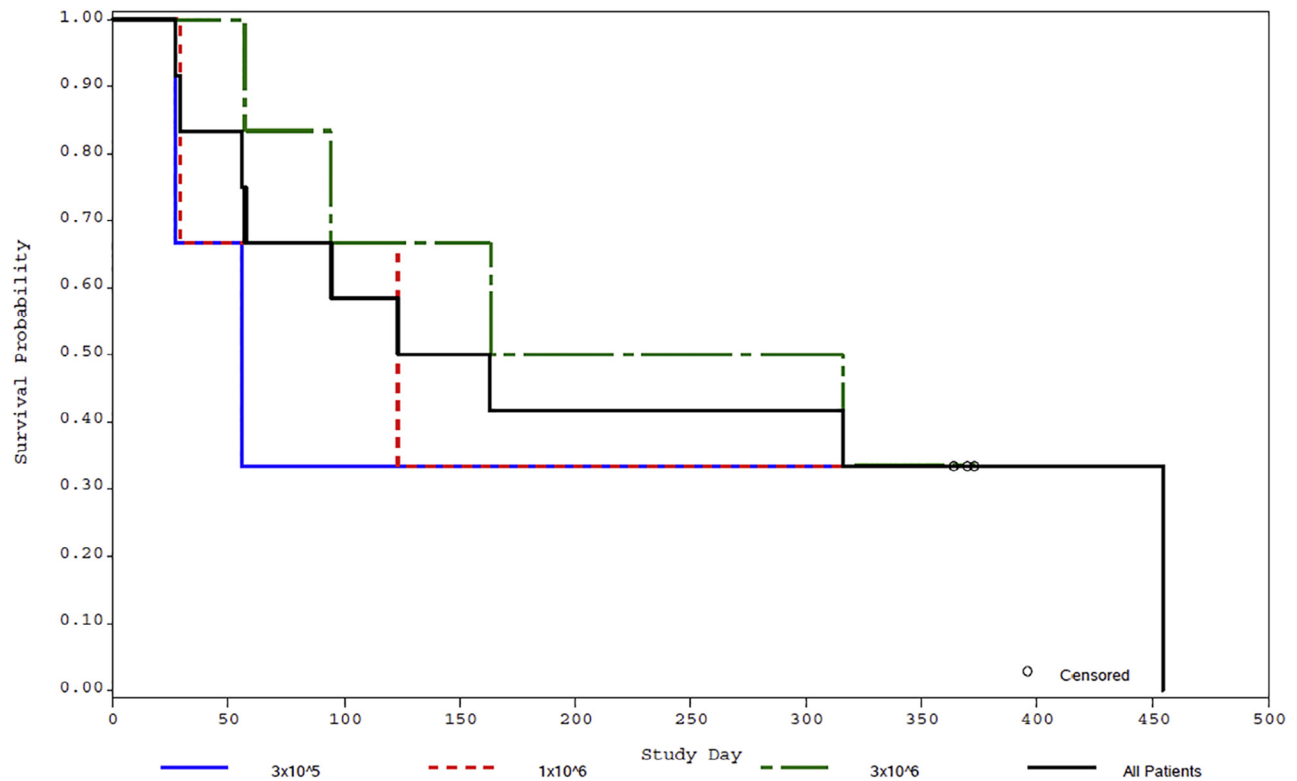Values are n (%). MedDRA indicates Medical Dictionary for Regulatory Activities.

Figure 1. Kaplan-Meier curve of RFS (Full Analysis Set). RFS was defined as the time from the date of CR1 to the date of relapse (per IWG response criteria for AML) or death due to any cause through the date cut-off of February 10, 2017. Dose level cohorts are indicated.

confirmation in larger clinical trials of patients with myeloid diseases.

Similar to other clinical trials of allogeneic HLA-haploidentical NK cells [10-14], GVHD, cytokine release syndrome, and neurotoxicity were not observed. Dose escalation was well tolerated, and the target cell dose was 3×10^6/kg, the maximum dose routinely obtainable from a single donor leukapheresis. NK cell chimerism levels at 7 or 14 days after infusion have been associated with CR after IL-2–activated HLA-haploidentical NK cell therapy [13]. Consistent with this finding, IL-12/-15/-18 preactivated NK cells were readily identified in both PB and BM at days 7 to 14 after

adoptive transfer into relapsed/refractory AML patients [14]. In a prior pilot trial using similarly generated CTV-1 lysate primed activated NK cells, PB chimerism was found to be discordant with the BM in at least 1 responding patient, raising the possibility that PB chimerism for CNDO-109-NK may not predict for antileukemia potential [16]. By 1 or both chimerism testing methods used (molecular short tandem repeat or anti-HLA mAbs), most patients (10/12) had evidence of donor chimerism on day 7 postinfusion and 3 had evidence of donor chimerism on or after day 14. In this allogeneic cell transfer system it was expected that recipient T cells would recover from fludarabine/cyclophosphamide suppression and

Table 4
Summary of RFS

RFS Definition/Analysis Population	Dose Group		
	3×10^5	1×10^6	3×10^6
RFS from date of CR1*			
Full Analysis Set			
N	3	3	6
Median (range)	105 (92-525)	156 (117-800+)	337 (176-677+)
Per-protocol			
N		3	5
Median (range)	309 (92-525)	156 (117-800+)	344 (183-677+)
RFS from date of CNDO NK cell treatment†			
Full Analysis Set			
N	3	3	6
Median (range)	56 (27-454)	123 (29-745+)	240 (57-560+)
Per-protocol			
N	2	3	5
Median (range)	241 (27-454)	123 (29-745+)	316 (94-560+)

* RFS duration, defined as the interval from the date of study product infusion CR1 to the date of relapse (according to IWG criteria for AML) or death.
† RFS duration also was analyzed using an updated definition of the interval from the date of study product infusion to the date of relapse (according to IWG criteria for AML) or death.

Table 5
Molecular and Flow Cytometry Chimerism

Days Postinfusion	Subject											
	1	2	3	4	5	6	7	8	9	10	11	12
Molecular chimerism: % donor DNA in NK—enriched MNCs												
Blood												
7	0	2	0	15	2	1	84	11	64	0	2	15
14	0	0	2	0	0	0	0	0	0	0	0	2
28	—	0	0	0	0	0	0	0	0	—	0	0
56	—	2	0	—	—	—	0	—	—	—	—	0
112	—	0	—	—	—	—	—	—	—	—	—	—
170	—	0	—	—	—	—	—	—	—	—	—	—
BM												
56	—	1	0	—	0	0	0	—	0	0	—	0
112	—	0	—	—	—	—	—	—	—	—	—	—
170	—	0	—	—	—	—	—	—	—	—	—	—
Flow cytometry chimerism: % donor DNA in NK—gated population												
Blood												
7	.40	.40		15.80			ND		66.20		7.03	
14	.09	.08		.00			.39		.00		2.37	
28		.00		.03			.03		.00		.85	
56		.01					.02		.00		.03	
112		.12										
170		.08										
BM												
56		.06					.02		.01		.04	
112		.26										
170		.58										

MNC indicates mononuclear cell; —, indicates Not Done (ND).

ultimately reject the allogeneic CNDO-109-NK. This expected lack of long-term persistence was observed in chimerism testing in this trial, and in this small sample set, the durability of NK cell chimerism was not correlated with RFS.

HLA-haploidentical NK cells are emerging as a promising immunotherapy approach for AML [21]. Multiple methods are being explored to enhance the potency of NK cells against leukemia or other cancer targets, including augmentation of NK cell functional capacity, enhanced targeting of NK cells to the leukemia target, and elimination of negative regulators of NK cell responses in the leukemia microenvironment [22]. For more than a decade short-term, high-dose IL-2 activation has been used to prime NK cell activity before adoptive transfer and alone has resulted in CRs in some AML patients (~30%) with active disease, with limited duration [10]. More recently, combined cytokine preactivation with IL-12, IL-15, and IL-18 has shown promise in differentiating long-lived NK cells with enhanced antileukemia properties, inducing CRs in ~50% of relapsed/refractory AML patients [14]. Cytokines (eg, IL-15–based therapeutics) [8,23] and activating receptor agonists (eg, anti-CD137 mAbs) [24,25] are also being tested in the clinic to maintain or promote ongoing in vivo NK cell responses. CNDO-109-primed NK cells exhibit short-term enhancement of normally NK cell–resistant tumors and likely use a mechanism of action distinct from cytokines receptors or CD137 [9]. Preclinical studies indicate that induction of CD69 and recognition of AML blasts by CNDO-109-primed NK cells requires CD15 and CD2 interactions [9,15,16]. Thus, strategies that use cytokine stimulation or activating receptors, combined with CNDO-109-NK priming, may further optimize the antileukemia capacity of primary donor NK cells. Similarly, a number of ex vivo expansion approaches are being evaluated to generate large numbers of NK cells for adoptive therapy, which may also combine with CNDO-109–based priming [21]. Finally, negative regulators of the NK cell antitumor response are emerging as therapeutic targets, including regulatory T cells [13] and myeloid-

derived suppressor cells [26] in the AML microenvironment or inhibitory receptors expressed or induced on NK cells. Combining the CNDO-109-NK tumor-priming based approach with such NK checkpoint blockade is also of interest for multimodality NK cell immunomodulation [22].

In summary, this trial establishes proof-of-concept that CNDO-109-NK cells may be readily manufactured in a scalable central processing site, viably cryopreserved, safely administered at doses of at least 3×10^6/kg, persist transiently in patients with myeloid malignancy, and have resulted in longer than expected CRs in high-risk AML patients treated in CR1. Thus, CNDO-109-NK represents a potential platform for HLA-haploidentical NK cell therapeutics. Future phase 2 clinical trials of 3×10^6/kg CNDO-109-NK are under development that will address their efficacy in myeloid diseases, and preclinical research that combines CND0-109 tumor priming with other NK cell immunomodulation strategies is warranted.
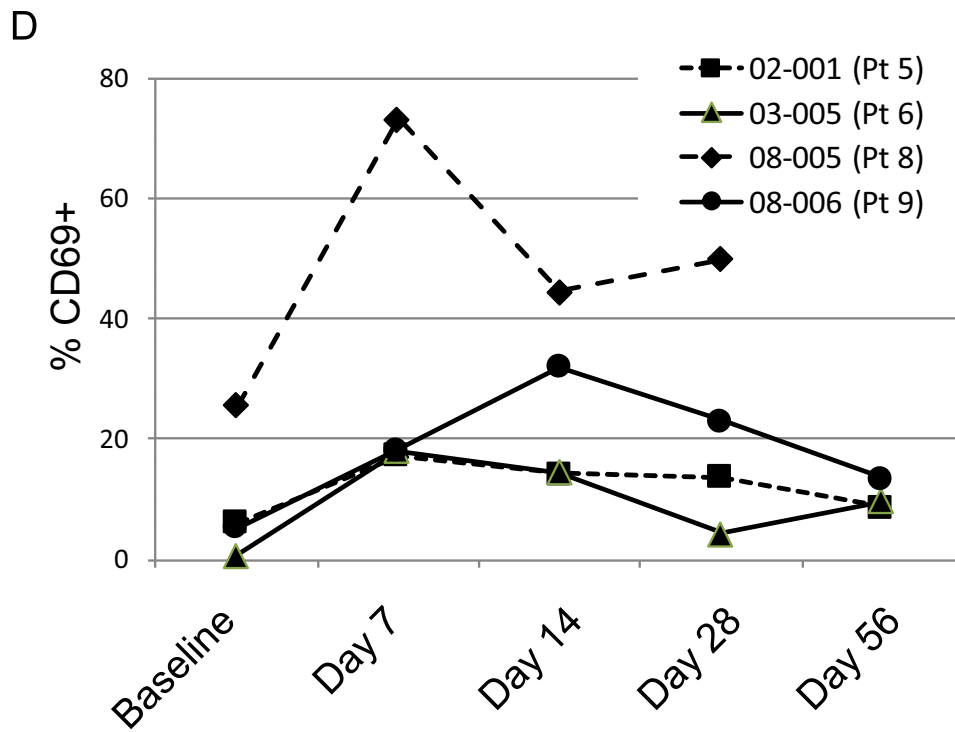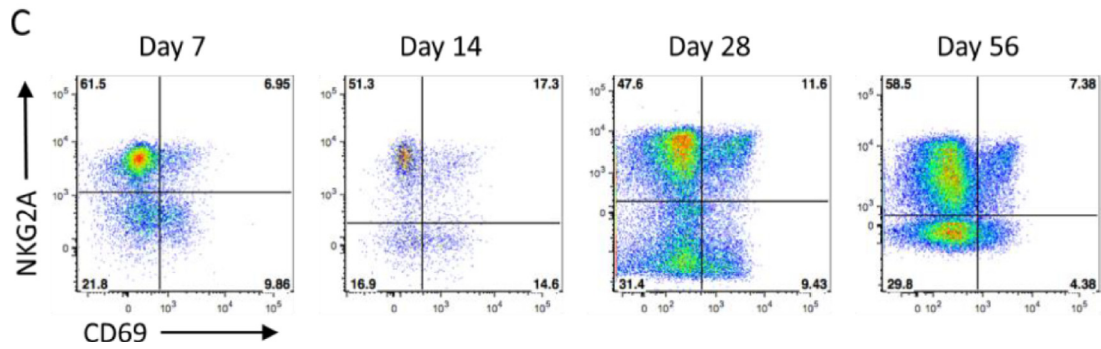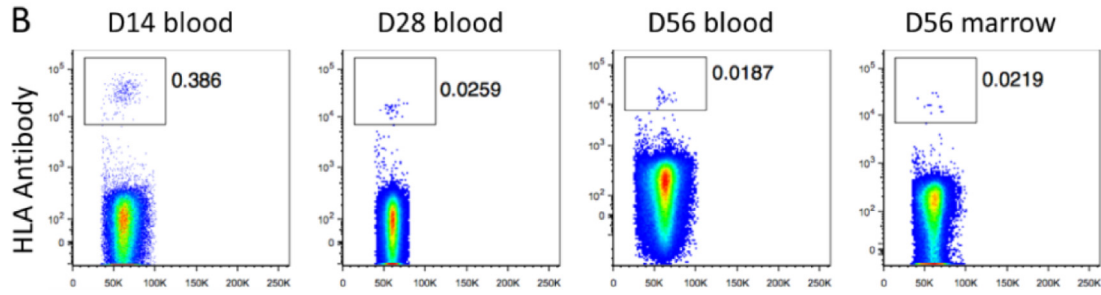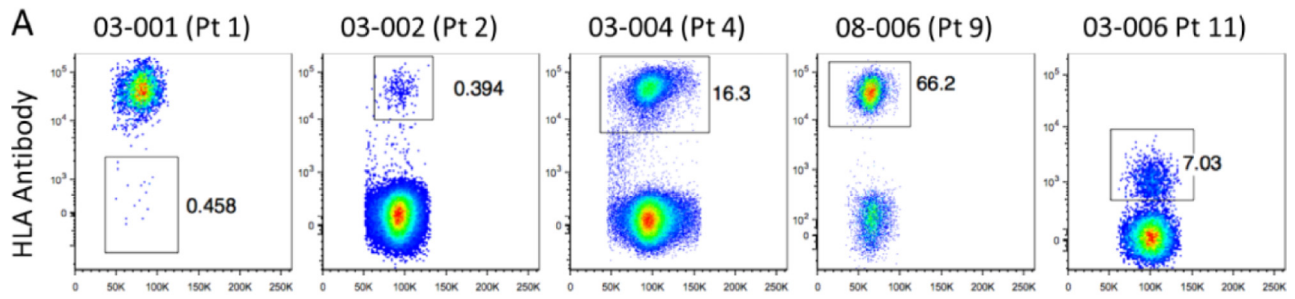
Figure 2. Donor-activated NK cells detected by flow cytometry chimerism and elevated CD69 expression on NK cells in the blood of 4 of 8 patients evaluated. All results in A to D are from plots gated on CD56+CD3− lymphocytes (NK cells). For 6 patients it was possible to evaluate chimerism in blood and BM samples using donor-specific anti-HLA mAbs. In A and B, rectangle gates include cells with the donor HLA type; numbers shown are the frequency of donor cells in the total NK cell population. (A) Circulating donor cells were detected by HLA antibodies in day 7 blood of 5 of 6 patients with an identifying HLA antibody. (B) The sixth patient, patient 08-004, had insufficient cells to perform flow cytometry chimerism analysis on day 7; flow chimerism analysis of subsequent samples detected donor cells by anti-HLA antibody as late as day 56 in the blood and BM. In 8 patients total NK cells in the blood (both donor and recipient) were evaluated for elevated expression of CD69. In C and D, numbers shown are the percent of NK cells. (C) Representative plots from patient 08-006 showing expression of CD69 versus NKG2A on total NK cells in the blood on days 7, 14, 28, and 56 postinfusion. (D) Fraction of total NK cells expressing CD69 in the blood of the 4 patients with elevated CD69 expression.

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