Phase 1 Clinical Investigation of Human Myeloid Progenitor Cells (CLT-008) as a Supportive Care Measure After Chemotherapy for Acute Myeloid Leukemia (AML)

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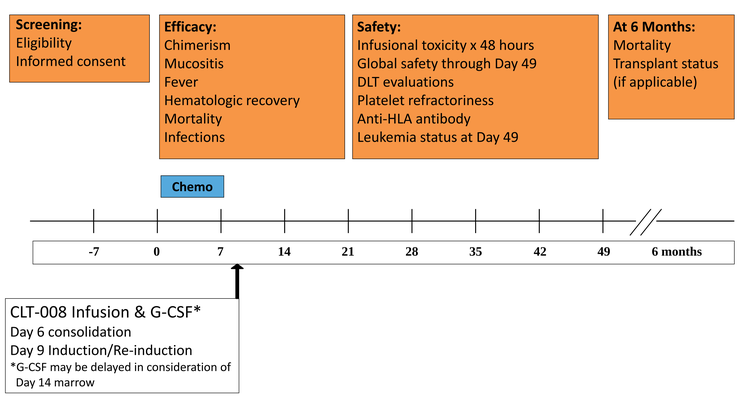
Introduction: Infection is the leading cause of death during induction therapy for acute myeloid leukemia (AML). Although the use of broad-spectrum antibiotics and antifungal agents has improved survival, infection-related death is an ongoing challenge. Myeloid growth factors reduce the duration of neutropenia, reduce days of neutropenic fever, and shorten hospital stay but have not been shown to improve survival. The myeloid progenitor cells that these growth factors stimulate are depleted by induction chemotherapy and cannot respond until they begin to regenerate weeks after initiation of induction chemotherapy. Accordingly, administration of allogeneic myeloid progenitor cells followed by G-CSF prior to autologous bone marrow recovery may reduce infection-related death and mitigate organ damage including mucositis. Unlike granulocyte transfusions, a cryopreserved myeloid progenitor product would be available "off-the-shelf", practical for infection and mucositis prophylaxis, and, because myeloid progenitors mature *in vivo*, the cells have not been damaged by storage.

The CLT-008 product is culture-derived from G-CSF-mobilized hematopoietic stem cells and can be cryopreserved for future infusion without HLA matching. CLT-008 is intended to engraft transiently and produce mature myeloid cells that migrate rapidly to tissues damaged by chemotherapy or radiation. In tissues, mature myeloid cells may mitigate the risk of bacterial or fungal infection for weeks, as demonstrated in pre-clinical murine models (Singh 2012; Bitmansour 2002; Bitmansour 2005; Arber 2005). Here we report a phase 1 trial evaluating the safety and preliminary efficacy of CLT-008 administered after induction or consolidation chemotherapy for AML.

Methods: A phase 1 dose-escalation study was conducted initially in subjects with AML, MDS, or ALL undergoing high-dose cytarabine-based consolidation (n=18) and in AML subjects undergoing induction or re-induction (n=27). Eligible subjects were age 18 or older and treated with either a high-dose or standard-dose cytarabine-based regimen. All 18 consolidation subjects and 27 induction/re-induction subjects received CLT-008. During induction, 9 additional non-randomly selected control subjects received G-CSF without CLT-008 cells. Chemotherapy was begun on Study Day 0. Subjects received CLT-008 on Study Day 6 in consolidation (10^6 or 10^7cells/kg) or on Study Day 9 or 10 in induction (10^7 or $3x10^7$ cells/kg). G-CSF 5 µg/kg was administered daily from the day of CLT-008 infusion until ANC greater than 500/µL for 3 consecutive days (Figure 1). Dose limiting toxicities (DLTs) were defined as any grade 3-5 infusion reaction, grade 4-5 organ toxicity not due to the primary malignancy, infection, pre-existing condition, or initiation of new chemotherapy, any grade 3-5 GVHD, or failure of autologous neutrophil recovery at Day 42 not clearly attributable to another cause. Dose-escalation followed standard 3+3 design followed by dose expansion at the maximum tolerated or maximal planned dose. Subjects were followed for 49 days for chimerism, fever, mucositis and platelet refractoriness. Clinical status was assessed at 6 months.

Figure 1: Overall Study Schematic for Consolidation and Induction/Re-induction



Results: Safety related results and chimerism are presented for all subjects. Efficacy results are presented for induction/re-induction subjects only.

Safety and Tolerability: Among the 45 currently evaluable subjects who received CLT-008, 1 experienced a DLT of grade 3 allergic infusion reaction which resolved promptly with steroids and antihistamine. There were no other DLTs and no GVHD of any grade. Severe adverse events that were grade 3 or grade 4 and judged possibly related to CLT-008 included preferred terms infusion-related reaction (also a DLT), febrile neutropenia, pyrexia, blood bilirubin increased, and hypotension (Table 1).

Potential clinical consequences from leukocyte alloimmunization were monitored and include immunologic refractoriness to platelet transfusion and engraftment failure of subsequent allogeneic HCT. Platelet refractoriness was observed in 3 of the 43 subjects treated with CLT-008 for whom data are available. Although no attempt was made to rigorously distinguish immunologic from non-immunologic platelet refractoriness, none of the 3 subjects had an increase in alloantibody to CLT-008. Among 40 subjects with 6-month follow-up data currently available, 20 had gone on to receive an allogeneic transplant. Of the 18 with follow-up data available, 17 successfully engrafted, and 1 died before engraftment was expected from causes unrelated to CLT-008.

Table 1: Grade 3 or 4 Adverse Events Occurring in 2 or More Subjects

Preferred Term	Induction/ Re-induction		Consolidation	Total
	7 + 3 N = 9 n (%)	HIDAC N = 18 n (%)		
Febrile neutropenia	5 (56)	10 (56)	11 (61)	26 (58)
Hypophosphatemia	1 (11)	4 (22)	0 (0)	5 (11)
Hypokalemia	0 (0)	3 (17)	0 (0)	3 (7)
Hypotension	0 (0)	2 (11)	1 (6)	3 (7)
Klebsiella bacteremia	0 (0)	0 (0)	3 (17)	3 (7)
Pyrexia	0 (0)	3 (17)	0 (0)	3 (7)
Urinary tract infection	1 (11)	0 (0)	2 (11)	3 (7)
Blood bilirubin increased	1 (11)	0 (0)	1 (6)	2 (4)
Diarrhea	0 (0)	2 (11)	0 (0)	2 (4)
Hypoxia	0 (0)	1 (6)	1 (6)	2 (4)
Neutropenia	1 (11)	1 (6)	0 (0)	2 (4)
Thrombocytopenia	0 (0)	0 (0)	2 (11)	2 (4)
Transfusion reaction	0 (0)	0 (0)	2 (11)	2 (4)

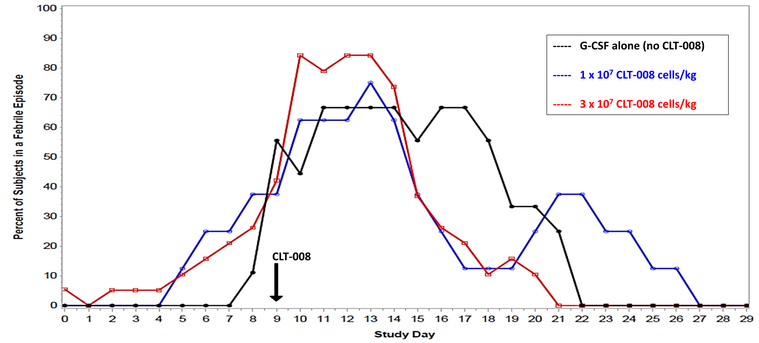
Chimerism: CLT-008-derived cells were detectable in the peripheral blood for 6 of 45 subjects at approximately 2-10% of total peripheral blood nucleated cells using the STR-PCR method; there was no clear increase in peripheral blood ANC resulting from this CLT-008-derived chimerism. One subject had 41% CLT-008 chimerism but was excluded from the analysis because the sample had been drawn soon after CLT-008 infusion.

Preliminary Efficacy: Among the 27 induction subjects receiving CLT-008 with G-CSF, there was a dose-dependent reduction in mucositis (WHO grade 2 or greater) and in duration of febrile episodes when compared to the 9 control subjects who received G-CSF without CLT-008 cells (Table 2 and Figure 2). A febrile episode begins with an oral temperature ≥38°C and is resolved when the maximum daily temperature is <37.5°C for 3 consecutive days (i.e. a minimum of 48 hours).

Table 2: Characteristics of the Induction/Re-induction Study Population

	G-CSF Alone (no cells)		1×10^7 CLT-008 cells/kg		3×10^7 CLT-008 cells/kg	
Chemotherapy regimen	7+3	HIDAC	7+3	HIDAC	7+3	HIDAC
n	3	6	6	2	3	16
Age, median (range)	68 (61-77)	58.5 (36-72)	61 (24-81)	67.5 (67-68)	69 (65-73)	60 (21-78)
Days with ANC <500/µl, median (range)	11 (11-23)	13 (8-34)	15 (11-36)	24 (8-40)	13 (11-15)	11.5 (9-35)
Response Complete remission Treatment failure Data pending	4 of 7 (57%) 2 of 7 (43%) 3		5 of 8 (62.5%) 3 of 8 (37.5%) 0		12 of 15 (80%) 3 of 15 (20%) 4	
Mucositis, WHO Grade ≥2 by Day 28	33%		13%		5%	
Days in febrile episode, mean	7.0		6.4		5.6	
Mortality at Day 49	0 of 9		0 of 8		1 of 19	
Mortality at 6 months	3 of 7 (43%)		3 of 7 (43%)		4 of 18 (22%)	

Figure 2: Proportion of Induction/Re-induction Subjects in a Febrile Episode by Study Day



Conclusions:

- CLT-008 is safe and well tolerated at administered doses in this study.
- Maximal tolerated dose was not reached.
- 1 DLT of grade 3 allergic reaction was observed at 3 x 10^7 cells/kg.
- No evidence of sequelae from alloimmunization was observed.
- Among the induction subjects treated at $3x10^7$ cells/kg, reduction in incidence and severity of mucositis and shortened duration of fever were observed even in the absence of consistently high-level peripheral blood chimerism for cells derived from CLT-008.
- Complete remission rate was similar across treatment cohorts.
- A randomized phase 2 study of an enhanced-potency iteration of the CLT-008 product with G-CSF *vs.* G-CSF alone after induction for untreated AML will compare infection-related outcomes and rates of mucositis with assessment of tissue-level chimerism.

References:
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