Clofarabine, high-dose cytarabine and liposomal daunorubicin in pediatric relapsed/refractory acute myeloid leukemia: a phase IB study

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Supplemental data

Supplemental methods

Methods S1. In-and exclusion criteria

Diagnosis and main criteria of inclusion (Dose level 1-4):

- 2nd relapse of AML
- refractory AML in 1st relapse (defined as ≥ 20% blasts in the bone marrow after the 1st course of standard re-induction therapy)
- 1st early relapse (relapse within one year from initial diagnosis) of AML
- ≤18 years old at initial diagnosis
- Lansky play score ≥ 60; or Karnofsky performance status ≥ 60
- Life expectancy 6 weeks
- Calculated creatinine clearance ≥ 90 ml/min/1.73m2 as calculated by the Schwartz formula for estimated glomerular filtration rate (GFR) where GFR (ml/min/1.73 m2) = k*Height (cm)/serum creatinine (mg/dl). k is a proportionality constant which varies with age and is a function of urinary creatinine excretion per unit of body size; 0.45 up to 12 months of age; 0.55 children and adolescent girls; and 0.70 adolescent boys.
- Liver function:

Serum bilirubin ≤1.5 × upper limit of normal (ULN)
Aspartate transaminase (AST)/alanine transaminase (ALT) 2.5 × ULN Alkaline phosphatase 2.5 × ULN

Other:

- Able to comply with scheduled follow-up and with management of toxicity.
- For female patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study
- Male and female patients must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations

Exclusion criteria (Dose level 1-4)

General conditions:

- Isolated extramedullary relapse, including isolated CNS-relapse
- Symptomatic CNS leukemia in case of combined relapse
- Relapsed/refractory acute promyelocytic leukemia (APL)
- Relapsed/refractory myeloid leukemia of Down Syndrome (ML DS)
- Other serious illnesses or medical conditions
- Current uncontrolled infection
- Evidence of fungal infection by:
  - Evidence of pulmonary infiltrates suggestive of a fungal infection at HR-CT (within 3 weeks prior to enrollment)
  - A positive Aspergillus serum test (galactomannan), according to local laboratory practice (within 3 weeks prior to enrollment)
- Evidence of cardiac dysfunction (shortening fraction below 28%)
- Pregnant or lactating patients

Prior or current history:

- Use of any anticancer therapy within 2 weeks before study entry. The patient must have recovered from all acute toxicities from any previous therapy (note: hematological toxicities do not need to be considered since the patient has overt leukemia).
- History of prior veno-occlusive disease (VOD)

- Hypersensitivity to cytarabine, clofarabine or liposomal daunorubicin

Concomitant treatments:

- Concomitant administration of any other experimental drug under investigation, or concurrent treatment with any other anti-cancer therapy other than specified in the protocol is not allowed.
- GCSF will not be used for priming and no routine GCSF support is allowed during the 1st course, except for life-threatening infections.

In case of non-symptomatic CNS-involvement, intrathecal therapy is allowed according to investigator's discretion. It is not allowed to give intrathecal therapy prior to treatment with clofarabine, as we do not know if this can be done without safety concerns. Hence, this should be delayed to day +7 of treatment, which will allow us to assess the CSF penetration of clofarabine (clofarabine CSF levels and early response). In case of neurotoxicity experienced during the IV treatment, the intrathecal may need to be further delayed

Inclusion main criteria for Dose Level 5

Initial work-up:

Newly diagnosed 1st relapse of AML: only patients with early relapses occurring within 1 year of initial diagnosis are eligible:

- ≤18 years old at initial diagnosis
- Lansky play score ≥ 60; or Karnofsky performance status ≥ 60
- Life expectancy ≥6 weeks
- Calculated creatinine clearance ≥90 ml/min/1.73m2 as calculated by the Schwartz formula for estimated glomerular filtration rate (GFR) where GFR (ml/min/1.73 m2) = k*Height (cm)/serum creatinine (mg/dl). k is a proportionality constant which varies with age and is a function of urinary creatinine excretion per unit of body size; 0.45 up to 12 months of age; 0.55 children and adolescent girls; and 0.70 adolescent boys.
- Liver function:
- Serum bilirubin ≤1.5 × upper limit of normal (ULN)
- Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 × ULN
- Alkaline phosphatase ≤2.5 × ULN

Other:

- Able to comply with scheduled follow-up and with management of toxicity.
- For female patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study
- Male and female patients must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations

Exclusion Criteria for Dose Level 5 only

General conditions:

- Isolated extramedullary relapse, including isolated CNS-relapse
- Symptomatic CNS leukemia in case of combined relapse
- Relapsed/refractory acute promyelocytic leukemia (APL)
- Relapsed/refractory myeloid leukemia of Down Syndrome (ML DS)
- Other serious illnesses or medical conditions
- Current uncontrolled infection
- Evidence of fungal infection by:
  - Evidence of pulmonary infiltrates suggestive of a fungal infection at HR-CT (within 3 weeks prior to enrollment)
  - A positive aspergillus serum test (galactomannan), according to local laboratory practice (within 3 weeks prior to enrollment)
- Evidence of cardiac dysfunction (shortening fraction below 28%)
- Pregnant or lactating patients

- Prior stem-cell transplant in CR1

Methods S2. Treatment

Clofarabine was administered intravenously in 2 hours (day 1-5); DNX in 1 hour (day 1, 3, 5), starting 30 minutes after the end of clofarabine; cytarabine was administered intravenously in 3 hours (day 1-5), starting 3 hours after the end of clofarabine. Intrathecal therapy was administered at day 6 with cytarabine for prophylaxis, or triple therapy (cytarabine and methotrexate and prednisolone) with age-adjusted dosages in case of central nervous system involvement.[2]

G-CSF was not allowed during chemotherapy and after the first course, unless indicated for life-threatening infections. The use of azoles was recommended to be interrupted during and 5 days after chemotherapy. The use of prophylactic antibacterial, antifungals, and antiviral agents was recommended according to each institution's guidelines. Broad-spectrum antibiotics with coverage for empiric gram-positive coverage was advised to initiate for patients with neutropenic fever. For patients with high peripheral blast counts (>100.000/uL) and/or evidence of disseminated intravascular coagulation was advised to keep platelet counts >20.000/uL and/or hemoglobin levels below 10mg/dl ~ 6 mmol/l.

Study treatment was continued until progressive disease, unacceptable toxicity or patient withdrawl, for a maximum of 2 courses. A 2nd course could only be given if there was no evidence of progressive disease. Subsequent stem cell transplantation (SCT) was left at the discretion of the treating physician.

Methods S3. Dose-limiting toxicities, Safety and Efficacy Evaluation

Adverse events (AEs) were graded according to Common Terminology Criteria for Adverse Events version 3.0. Dose Limiting Toxicities (DLT) were defined as grade 3 or 4 non-hematological AEs and hematological AEs lasting longer than 42 days, limited to the first course, and at least possibly drug-related, with some exceptions.

DLTs consisted of grade ≥3 AEs despite appropriate medical management; a laboratory abnormality grade 4 or grade 3 lasting ≥7 days and requiring discontinuation/interruption/dose-reduction; and any clinically important ≥grade 2 toxicity requiring discontinuation/interruption for ≥7 days or dose- reduction. Hematological DLTs consisted of grade 3 or 4 myelosuppression, worsening from baseline, and lasting ≥42 days in responding patients without evidence of persistent leukemia. Myelosuppression/pancytopenia due to persistent leukemia, grade 3 febrile neutropenia, grade 3 subsequently controlled nausea/vomiting, transient grade 3 transaminase elevations, drug fever, anorexia and alopecia were not considered as DLTs.

Response evaluation (bone marrow, peripheral blood and lumbar punction) were performed at day +21 (or repeated in case of persisting aplasia) after the first and second course. In case of persisting aplasia without evidence of leukemia at day +28, the next evaluation was delayed to day +42, to neutrophil recovery or to progressive leukemia, whichever occurred first. Responses were evaluated by morphological examination and flow cytometry. Morphological determination of response (complete remission [CR], complete remission with incomplete blood count recovery [CRi], no evidence of leukemia [NEL] and partial response [PR]) was centrally reviewed under supervision of DR at the laboratory of the AML-BFM Study Group (Table S1). Overall response rate (ORR) was defined as CR, CRi, NEL and PR. The response rates were determined by dose-level, and by early versus late relapse. Where appropriate also rates of CR/CRi were given. Efficacy variables included hematologic response, time to and duration of response, EFS and OS, and the number of patients able to undergo SCT.

Methods S4. Pharmacokinetics

Plasma and CSF samples were extracted by methanol solution containing the internal standard cladribine. Reconstituted samples were separated on Accela UPLC system (Thermo Scientific, UK), fitted with Ascentis Express (Sigma Aldrich, UK), C18, 2.7 µm, 300Å, 4.6 × 100 mm HPLC column using a gradient elution of water and acetonitrile containing 0.1% formic acid (Fisher Scientific, UK) at a flow rate of 300 µl/min. A triple stage quadrupole Vantage mass spectrometry system (Thermo

Scientific, UK) equipped with heated electrospray (HESI) ion source was used to detect the analytes using the optimum transitional daughter ions mass as follows: Clofarabine m/z 304.0 $\rightarrow$ 170.0 (collision energy 20 V) and cladribine m/z 286.0 $\rightarrow$ 170.0 (collision energy 15 V). Clofarabine PK parameters were derived on day 1 and 5 in plasma and CSF. Plasma and CSF concentrations were analyzed using a validated novel LC-MS/MS method (Methods S3). Clofarabine PK data were analyzed using non-compartmental analysis (NCA) method and plotted using GraphPad Prism software version 5.03.

Methods S5: Statistical Analysis

EFS was defined as the time between enrolment in the study and first event including relapse, death of any cause, failure to achieve remission (non-responders were considered as an event at time 0) and second malignancy. OS was defined as time between the date of enrolment and death.

Supplemental tables

Table S1. Response Definitions

Morphologic Complete remission (CR)	Morphologic leukemia-free state, an absolute neutrophil count of 1,000/µL and platelets of 80,000/µL, no residual evidence of extramedullary leukemia. The patient must be independent of transfusions.
Morphologic Complete remission with incomplete blood count recovery (CRi)	CR except for residual neutropenia (<1,000/µL) or thrombocytopenia (<80,000/µL).
No evidence of leukemia (NEL)	Less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells. Less than 5% of cells with a unique phenotype (measured by flow cytometry) identical to the cell population, which was found in the pretreatment specimen. No blasts with Auer rods or persistence of extramedullary disease.
Partial response (PR)	Decrease of at least 50% in the percentage of blasts to 5-25% in the bone marrow aspirate.
Stable disease (SD)	All patients who do not fulfill criteria for PD or CR.
Progressive disease (PD)	Increase of at least 50% in the percentage of blasts in the bone marrow aspirate or in peripheral blood, or occurrence of new disease localizations.
Treatment failure	Patients for whom treatment has failed to achieve less than a PR.
Overall Survival (OS)	Time from first dose of study treatment to date of death. Subjects lost to follow-up will be censored on the last date the subject was known to be alive.
Event free survival (EFS)	Time between diagnosis and first event (relapse, death of any cause, failure to achieve remission or second malignancy) or date of last follow-up. Patients who were classified as treatment failures will be considered as failures at time zero.
Early death	Death during the first 3 weeks of treatment.
Relapse	Reappearance of leukemic blasts in the peripheral blood, or 5% blasts in the bone marrow not attributable to any other cause. The reappearance or development of cytological proven extramedullary disease also indicates relapse.
Early relapse	Relapse within 12 months from initial/1st relapse diagnosis.
Late relapse	Relapse later than 12 months from initial/1st relapse diagnosis.

Table S2. Pharmacokinetic sampling schedule

	Day	Pre-dose	2 hours post Clofarabine infusion	5 hours post Clofarabine infusion prior to cytarabine	24 hours after the last Clofarabine infusion
Clofarabine	1	PB	PB	PB	
Clofarabine	5	PB	PB	PB	
Clofarabine	6				PB and CSF

Abbreviation: PB, peripheral blood; CSF, cerebrospinal fluid

Table S3. SAEs

	Total	Category						
		Febrile Neutropenia	Infection	Gastrointestinal	Skin reaction	Capillary leak	Hypotension	Hypoxia
Reason AE is serious								
Total Nr of SAEs	34	18	9	3	1	1	1	1
Death	1 (3%)	1	-	-	-	-	-	-
Life Threatening	6 (18%)	1	2	-	-	1	1	1
(Prolongation of) hospitalization	26 (76%)	16	6	3	1	-	-	-
Other medically important condition	1 (3%)	-	1	-	-	-	-	-
Outcome of SAE								
Resolved completely or with sequelae	25 (74%)							
Resolved with sequelae	1 (3%)							
Ongoing	5 (15%)							
Death	2 (6%)							
Ongoing at death	1 (3%)							

Abbreviations: AE, adverse event; SAE, severe adverse event.

Table S4. Response after cycle 1 by assigned dose level.

	All patients (n=34)	DL 1 (n=4)	DL 2 (n=3)	DL 3 (n=12)	DL 4 (n=10)	DL 5 (n=5)
Morphologic response						
CR	5 (15%)				2	3
CRi	15 (44%)	1	2	6	5	1
PR	1 (3%)				1	
NEL	0 (0%)					
SD	6 (18%)	1		3	1	1
PD	3 (9%)	2		1		
Treatment failure	1 (3%)		1			
Non-evaluable	3 (9%)			2	1	

Abbreviations: DL, dose-level; CR, complete remission; CRi, Morphologic Complete remission with incomplete blood count recovery; NEL, no evidence of leukemia; PR, Partial response; SD, Stable disease; PD, Progressive disease.