Clofarabine with topotecan, vinorelbine, and thiotepa reinduction regimen for children and young adults with relapsed AML

Tracking no: ADV-2021-005753R1

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Abstract:
Effective reinduction regimens are needed for children with relapsed and refractory acute myeloid leukemia (AML) as outcomes remain poor. Therapeutic options are limited in this heavily pre-treated patient population, many of whom have reached lifetime recommended doses of anthracycline chemotherapy. The development of effective non-anthracycline based salvage regimens is crucial to these patients who are at significant risk of life-threatening cardiotoxicity. We previously reported results of a phase 2 trial of a clofarabine-based regimen with topotecan, vinorelbine, and thiotepa (TVTC) in patients with relapsed acute leukemias. Here we report on an expanded bicenter cohort of 33 patients, <25 years of age, with relapsed/refractory (R/R) AML treated with up to 2 cycles of the TVTC reinduction regimen from 2007 to 2018. The overall response rate (ORR), defined as complete remission (CR) or CR with partial recovery of platelet count (CRp), was 71.4% (95%CI: 41.9 to 91.6%) for those patients in first relapse (n=14) and 47.4% (95%CI: 24.4 to 71.1%) for patients in 2nd or greater relapse or refractory disease. Responses were seen across multiple high risk cytogenetic and molecular subtypes, with 84% of responders successfully bridged to allogeneic stem cell transplantation. The 5-year OS for patients in first relapse was 46.2% (95%CI: 19.1 to 73.3%) and 50.0% (95%CI: 26.9 to 73.1%) for patients who responded to TVTC. For pediatric and young adult patients with R/R AML, TVTC reinduction compares favorably with currently used salvage regimens and warrants further exploration.

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS substantially contributed to conception and design of the work. KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS contributed to the acquisition of data, analysis and interpretation of the data. KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS assisted with drafting and provided critical review and revision of the manuscript. All authors approved the final version of the manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Emails to the corresponding author.

Clinical trial registration information (if any):
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Short running title: TVTC for relapsed/refractory AML in children

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Abstract word count: 239
Text word count: 2469
Figures/tables: 1/3
References: 38

Presented as an abstract, “Clofarabine with Topotecan, Vinorelbine, and Thiotepa (TVTC) in Children and Young Adults with Relapsed or Refractory Acute Myeloid Leukemia”, presented at American Society of Hematology, Dec 2018; Blood 2018; 132 (Supplement 1): 79.
doi: https://doi.org/10.1182/blood-2018-99-115185.
Abstract:

Effective reinduction regimens are needed for children with relapsed and refractory acute myeloid leukemia (AML) as outcomes remain poor. Therapeutic options are limited in this heavily pre-treated patient population, many of whom have reached lifetime recommended doses of anthracycline chemotherapy. The development of effective non-anthracycline based salvage regimens is crucial to these patients who are at significant risk of life-threatening cardiotoxicity. We previously reported results of a phase 2 trial of a clofarabine-based regimen with topotecan, vinorelbine, and thiotepa (TVTC) in patients with relapsed acute leukemias. Here we report on an expanded bicenter cohort of 33 patients, <25 years of age, with relapsed/refractory (R/R) AML treated with up to 2 cycles of the TVTC reinduction regimen from 2007 to 2018. The overall response rate (ORR), defined as complete remission (CR) or CR with partial recovery of platelet count (CRp), was 71.4% (95%CI: 41.9 to 91.6%) for those patients in first relapse (n=14) and 47.4% (95%CI: 24.4 to 71.1%) for patients in 2nd or greater relapse or refractory disease. Responses were seen across multiple high risk cytogenetic and molecular subtypes, with 84% of responders successfully bridged to allogeneic stem cell transplantation. The 5-year OS for patients in first relapse was 46.2% (95%CI: 19.1 to 73.3%) and 50.0% (95%CI: 26.9 to 73.1%) for patients who responded to TVTC. For pediatric and young adult patients with R/R AML, TVTC reinduction compares favorably with currently used salvage regimens and warrants further exploration.

Key points:

1) The ORR for children and young adults with AML receiving TVTC in first relapse was 71.4%.
2) Eighty-four percent of responders were bridged to HSCT without further anthracycline exposure with 56.2% demonstrating long-term survival
Introduction

Newly diagnosed children with acute myeloid leukemia (AML) have long term event free survival rates ranging from 50-63% when treated with contemporary frontline regimens [1-4]. Children with relapsed disease, albeit a heterogenous patient population, carry a poor prognosis despite intensive salvage regimens [5, 6]. Among survivors of relapsed AML, over one-third will develop late cardiotoxicity from cumulative anthracycline containing frontline and salvage therapy regimens [7, 8]. There is an unmet need for effective and minimally cardiotoxic salvage regimens for children with relapsed AML.

Clofarabine is a deoxyadenosine analog, similar in structure to fludarabine and cladribine, but modified to enhance cytotoxic activity as well as stability [9]. Clofarabine was granted FDA approval for the treatment of relapsed acute lymphoblastic leukemia in 2004 following the results of a Phase 2 single-agent study [10].

We previously reported the results of a phase 1 and phase 2 trial of a clofarabine-based regimen with topotecan, vinorelbine, and thiopeta (TVTC) in a cohort of patients with relapsed acute leukemias [11, 12]. In the phase 2 study, 8/12 (67%) patients with relapsed or refractory (R/R) AML achieved a complete response (CR) or complete response with incomplete platelet recovery (CRp). Herein we describe the outcomes of an expanded retrospective cohort of 33 patients with relapsed or refractory (R/R) AML treated with the TVTC regimen.

Methods

Patients

Children and young adults less than 25 years of age with R/R AML were included in this bi-center retrospective analysis from 2007-2018. This included 11 AML patients treated on a previously published phase 2 clinical trial [12]. Patients were included from both Memorial Sloan Kettering Cancer Center (MSKCC) and University of California San Francisco Benioff Children’s Hospital of Oakland (BCHO). The institutions’ review boards approved the study and granted waivers of consent. Patients with R/R AML were defined as those having at least 5% leukemic burden in the bone marrow by morphologic assessment and those who were treated with at least one cycle of TVTC were included in the analysis. Patients with combined medullary relapse with central nervous system (CNS) involvement were included as well as those patients who received a prior hematopoietic stem cell transplant (HSCT). A total of 33 patients were identified who met the above criteria. The population was further stratified into 2 groups; Group A (n=14) being defined as those in 1st relapse (after having previously achieved a remission) with no additional re-induction attempt and Group B (n=19) as all other patients, including patients in 2nd or greater relapse, in 1st relapse and refractory to at least one re-induction attempt, or primary refractory disease. Patients with therapy-related AML, mixed phenotype acute leukemia, and myeloid leukemias of Down syndrome were excluded from this analysis. Patients with previous clofarabine exposure were not included. Total anthracycline exposure was calculated using a conversion multiplier of 4 daunorubicin equivalents for mitoxantrone per the Children’s Oncology Group (COG) guidelines [13]. Genetic prognostic markers were identified based on the risk stratification for cytogenetic abnormalities in the ongoing COG AAML1831 trial for de novo AML.[14, 15].

Treatment and Response Assessment
Patients received the phase 2 recommended schedule of the TVTC regimen as follows: topotecan 1 mg/m²/day (120 hour continuous infusion, Days 0-4), vinorelbine 20 mg/m²/dose (Days 0, 7, 14), Thiotepa 15 mg/m²/dose (Day 2), and clofarabine 40 mg/m²/day (Days 3-7), see Supplemental Fig 1. Patients >21 years of age or pediatric patients receiving a 2nd cycle of TVTC were treated with reduced clofarabine dosing of 30mg/m²/day. Dexamethasone was given on Day 3 prior to starting Clofarabine to reduce the risk of capillary leak syndrome. Granulocyte colony stimulating factor (GCSF) was administered until signs of neutrophil recovery. Response was assessed through bone marrow (BM) evaluation and was performed at the point of hematologic recovery defined as absolute neutrophil count (ANC)>0.5 K/mcL and platelet count > 75 K/mcL. Outcomes of interest were defined as the following: overall response rate (ORR) as defined by complete remission (CR) of bone marrow blasts to <5% by morphology and normal count recovery or CR without platelet recovery (CRp) after up to 2 cycles of TVTC. Minimal residual disease (MRD) was assessed by multiparameter flow cytometry using the “different from normal” method as previously described with a threshold for positivity of 0.05% [16, 17]. In the absence of multiparameter flow, alternate methods of MRD assessment, including quantitative reverse transcription PCR (RT-qPCR) for inv(16) and t(8;21), and PCR for FLT3-ITD detection were used.

Statistical Analysis

Overall survival was defined as time from the initiation of TVTC to death from any cause. Patients alive were censored at their date of last follow-up. The 95% confidence intervals (95%CI) for the response rates were calculated using the exact method (Pearson-Klopper method). In order to assess the OS by response, a landmark analysis was conducted, with a landmark time at 2 months, assessing the impact of a response in the first 2 cycles.

Results

A total of 33 patients with R/R AML treated with the TVTC regimen were identified between the two centers (MSKCC n=31; BCHO n=2). Fourteen patients were treated in first relapse (Group A). Nineteen patients were treated in second or greater relapse or with relapsed/refractory or primary refractory disease (Group B). Patient characteristics of the entire cohort are shown in Table 1. The median age at the start of TVTC was 11 years old (range 1-24). All patients had an isolated BM relapse except for two patients who had a combined BM and CNS2 relapse. Both patients received additional weekly intrathecal treatment during therapy. Four patients in Group A (28.6%) received prior allogeneic HSCT and five (26.3%) in Group B. In those patients who received TVTC in first relapse, 7 had an early relapse (within a year from diagnosis) and 7 patients relapsed more than 1 year from diagnosis [18].

The ORR for patients in Group A and Group B was 71.4% (10/14; 95%CI: 41.9 to 91.6%) and 47.4% (9/19; 95%CI: 24.4 to 71.1%), respectively. All responses were seen after one cycle of TVTC except in a single patient who achieved CR following a second cycle of TVTC. The distribution of prognostic features by relapse subgroups, including cytogenetic and molecular findings, are presented in Tables 2 and 3. Patients in both Groups A and B presented with high-risk cytogenetic or molecular features. Responses were seen across multiple high-risk cytogenetic subtypes, but only 1 response seen among patients with
monosomy 7 or *ETV6* fusions (1/5). The cohort included 6/31 (19%) patients with favorable cytogenetics (4 in Group A and 2 in Group B), for whom the ORR was 100%. See Supplemental tables 1 and 2 for detailed individual patient characteristics of the 2 relapse subgroups.

The 5-year OS of patients in Group A and Group B was 46.2% (95%CI: 19.1 to 73.3%) and 31.6% (95%CI: 10.7 to 52.5%) (*Fig 1A*), respectively. Patients who responded to TVTC had a 5-year OS of 50.0% (95%CI: 26.9 to 73.1%) versus 25% for non-responders (95%CI: 0.5 to 49.5%) (*Fig 1B*). Of the 19 responders to TVTC in the entire cohort, 16 (84.0%) proceeded to receive allogeneic HSCT. The other 3 patients had disease recurrence prior to HSCT. The 5-year OS of responders who proceeded to allogeneic HSCT (n=16) was 56.2% (95%CI: 31.9 to 80.6%) (*Fig 3C*), which included patients from Group A and B (n=9 and 7, respectively). Of the 7 patients who died following HSCT, 4 were due to relapsed disease, and 3 from transplant related toxicities.

MRD data following treatment with TVTC was available in 12 of the 19 responders. Eight of 12 (67%) patients were MRD negative after one cycle of TVTC by either flow cytometry or RT-qPCR. All 8 patients who achieved MRD negativity proceeded to transplant and are alive without relapse at their last follow-up. Of the 4 patients with MRD positive disease, all proceeded to HSCT but eventually died from progressive disease within 6 months.

**Discussion**

The thiotepa, vinorelbine, and topotecan (TVT) backbone was originally developed to provide an anthracycline-free chemotherapeutic regimen of novel active agents to minimize risk of drug resistance. These agents used here have been shown in earlier studies to have single agent tumor activity in patients with refractory leukemias and activity when combined [19-23]. An initial study of TVT combined with dexamethasone alone or with dexamethasone and gemcitabine in children and young adults with relapsed acute leukemia reported a CR rate of 25% and 36% respectively [23]. Following a phase 1 study demonstrating the safety of adding clofarabine to the TVT backbone, a phase 2 study of TVTC reported an ORR as defined by CR or CRp in 8/12 (67%) children and young adults with R/R AML [12]. In this retrospective analysis, we sought to further explore the efficacy of TVTC in an expanded cohort of patients with R/R AML.

Anthracycline related cardiotoxicity is well established as a frequent consequence in survivors of childhood AML [24]. The cumulative incidence of late onset cardiotoxicity has been estimated to be over 25% in long term survivors of AML [7]. The incidence is significantly higher in survivors of relapsed AML [8]. Recent studies have also identified mitoxantrone, an agent frequently used in frontline or relapsed AML regimens, to be associated with significantly higher long-term cardiotoxicity risk than previously recognized [25].

Several strategies have been employed to reduce anthracycline related cardiotoxicity. The use of the antioxidant scavenger dexrazoxane in patients receiving frontline anthracycline containing therapy on the Children Oncology Group’s (COG) AAML1031 study led to smaller reductions in ejection and shortening fractions in treated patients [26]. Based on these results, dexrazoxane is administered for all patients receiving standard anthracyclines on the ongoing COG AAML1831 trial for children with newly
diagnosed AML. Liposomal preparations of daunorubicin have also been studied in multiple relapsed AML trials for children in an attempt to reduce anthracycline associated cardiotoxicity [6, 27]. Cooper et al in AAML1421 reported a 76% CR/CRp/CRi rate after one cycle of CPX-351 (now approved as Vyxeos), a liposomal preparation of daunorubicin and cytarabine, in children with AML in first relapse, with CR rate similar to patients treated with TVTC in this study (71.4%). In AAML1421, all evaluable patients with grade 2 or higher echocardiographic evidence of left ventricular systolic dysfunction had recovery of ejection fractions to >50%. CPX-351 is currently being evaluated in the frontline setting on COG AAML1831 to establish the short and long term cardioprotective benefits compared to standard daunorubicin. Finally, Rubnitz et al. recently published the results of the St Jude Children’s Research Hospital AML08 study which randomized children with newly diagnosed AML to receive a first round of induction with clofarabine and cytarabine instead of standard induction with cytarabine, daunorubicin, and etoposide [28]. There was no statistically significant difference in 3 year EFS or OS between the two arms, suggesting that anthracycline use may be safely reduced in children with newly diagnosed AML in an attempt to reduce short and long term cardiotoxicity.

The activity of clofarabine in relapsed childhood AML has been studied as both a single agent and in combination with multiple chemotherapeutic agents. Clofarabine demonstrated modest single agent activity in a phase 2 study wherein 1/42 (2%) patients achieved CR and 10/42 (24%) had a partial response. Patients were heavily pretreated in this study with 36/42 children having received 2 or more prior induction regimens [29]. A phase 1 study [30] and two relatively small studies of clofarabine combined with cyclophosphamide and etoposide in children with multiply relapsed AML reported CR/CRp rates close to 30% [31, 32]. The phase 2 portion of COG AAML0523 evaluated the efficacy of clofarabine with cytarabine for children in first relapse or refractory to induction. The combination resulted in a CR/CRp rate of 48% and a 3-year OS of 46% for responders [33]. In a phase 1/2 study investigating GCSF priming, high-dose cytarabine, and clofarabine (GCLAC), CRs were seen in 21 of the 46 evaluable patients (46%) [34]. The Innovative Therapies for Children with Cancer (ITCC) consortium conducted a phase 1B study of clofarabine combined with liposomal daunorubicin for children with R/R AML. Of the 31 patients evaluable for efficacy, 68% responded with CR/CRi following one cycle of therapy. When evaluated by disease phase, 87% of patients with early first relapse responded compared to 50% in second or greater relapse. The 2 year OS was 48% [35].

The toxicities of TVTC have been described in the phase 1 and 2 publications of the regimen, and included a substantial increase of high grade infections. In 2012, following these results, there was universal implementation of broad-spectrum antimicrobial prophylaxis for patients receiving this regimen at MSKCC. In this expanded retrospective cohort, there was a reduction in serious adverse events seen from the published phase 2 study of TVTC, with 22 patients developing grade 3 or greater febrile neutropenias (66.7%) and 5 documented bacteremias (15.1%). These findings are comparable to other studies of intensive salvage chemotherapy regimens for R/R AML [5, 36, 37]. One patient developed an abdominal mucormycosis infection. One patient developed bone marrow aplasia and died due to sepsis 45 days after receiving TVTC.

The outcomes reported herein of 33 children and young adults with R/R AML represent a substantial expansion of the cohort previously described in the phase 2 study of TVTC. The results compare
favorably to other published salvage regimens, both non-anthracycline regimens and those employing anthracyclines (with or without liposomal preparations). The ORR for patients in first relapse was 71.4%, with significant response rates demonstrated in patients with high-risk features, such as those with unfavorable cytogenetics as well with relapses less than one year after achieving remission. Furthermore almost half of all patients with second or greater relapse or primary refractory disease responded to TVTC. The regimen was successful as a bridge to transplant for the vast majority of responders, with a long term overall survival nearing 50% for patients in 1st relapse.

Of note, five patients with FLT3-ITD were treated with TVTC, four of whom did not respond and one had a brief response but ultimately died of progressive disease before being able to receive a HSCT. These patients were treated at a time when targeted FLT3 kinase inhibitors were not as readily available for children with relapsed AML. The poor outcomes of these 5 patients is not unexpected given the known poor response rates of children with FLT3-ITD relapsed AML treated with reinduction chemotherapy regimens [38]. Contemporary strategies for these patients usually incorporate FLT3 directed targeted therapies, and these patients are largely ineligible for trials utilizing chemotherapy alone. Limitations of this analysis are related to it being a retrospective study with the majority of patients treated at a single institution. Furthermore, MRD evaluation was unavailable for a significant proportion of the patient population. However these encouraging results in an expanded cohort of 33 patients warrant further evaluation of TVTC as a backbone regimen for relapsed AML, with particular potential utility in children who cannot tolerate additional anthracycline exposure.
Data sharing statement: For data sharing, contact the corresponding author: ramaswak@mskcc.org.

Acknowledgments: The authors acknowledge support from the NCI Cancer Center Support Grant P30 CA008748. The authors have no competing interests. The authors thank Joseph Olechnowicz, editor, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, for editorial assistance.

Authorship contributions: Author Contributions: KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS substantially contributed to conception and design of the work. KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS contributed to the acquisition of data, analysis and interpretation of the data. KR, PS, AKA, CF, AM, MR, TT, NAK, MLS, and NS assisted with drafting and provided critical review and revision of the manuscript. All authors approved the final version of the manuscript.

Disclosure of conflicts of interest: The authors have no related conflicts of interest.
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Figure Legends:

Figure 1. Outcomes of relapsed/refractory patients with AML treated with TVTC A) 2-year overall survival (OS) of patients in Group A vs Group B; C) OS by response to TVTC; D) 5-year OS of responders who proceeded to allogeneic HSCT (n=16).
Table 1: Study Population Characteristics

| Characteristic                                      | N = 33¹ |
|----------------------------------------------------|---------|
| Age at start of TVTC (years)                       | 11.0 [1–24] |
| Sex                                                |         |
| F                                                   | 17 (51.5%) |
| M                                                   | 16 (48.5%) |
| BM blasts, %                                        | 32 [10–99] |
| Prior HSCT                                          |         |
| N                                                   | 24 (72.7%) |
| Y                                                   | 9 (27.3%)  |
| Prior Anthracycline (cumulative dose mg/m²)         | 342 [60-492] |

| Group A                                             | N=14    |
|-----------------------------------------------------|---------|
| Length of CR1                                       |         |
| <180 days                                           | 1 (7.1%) |
| 180-365 days                                        | 6 (50%) |
| >365 days                                           | 7 (42.9%) |
| Prior HSCT                                          | 4 (28.6%) |

| Group B                                             | N=19    |
|-----------------------------------------------------|---------|
| Prior HSCT                                          | 5 (26.3%) |

¹Statistics presented: median [minimum – maximum]; n (%)  
TVTC, topotecan, vinorelbine, thiotepa, clofarabine;  
HSCT, hematopoietic stem cell transplantation  
Group A = 1st relapse  
Group B = 2nd or greater relapse/refractory/primary refractory
Table 2: Group A Characteristics

| Characteristic                  | Responders (n=10) | Non-responders (n=4) |
|---------------------------------|-------------------|----------------------|
| **Cytogenetic/Molecular Features** |                   |                      |
| Normal                          | 1                 | 0                    |
| Favorable inv(16), t(8;21)      | 4                 | 0                    |
| 11q23                           | 0                 | 1                    |
| Del5q/Monosomy 5                | 1                 | 0                    |
| Monosomy 7                      | 0                 | 1                    |
| FLT3-ITD                        | 1                 | 2                    |
| Other                           | 3                 | 0                    |
| **Prior HSCT**                  |                   |                      |
| N                               | 9 (90%)           | 1 (25%)              |
| Y                               | 1 (10%)           | 3 (75%)              |

Table 3: Group B Characteristics

| Characteristic                  | Responders (n=9) | Non-responders (n=10) |
|---------------------------------|-------------------|-----------------------|
| **Cytogenetic/Molecular Features** |                   |                       |
| Normal                          | 1                 | 0                     |
| Favorable inv(16), t(8;21)      | 2                 | 0                     |
| 11q23                           | 2                 | 0                     |
| Del5q/Monosomy 5                | 1                 | 1                     |
| Monosomy 7                      | 1                 | 2                     |
| FLT3-ITD                        | 0                 | 2                     |
| NUP98-NSD1                      | 1                 | 0                     |
| DEK-NUP214                      | 1                 | 0                     |
| ETV6 rearrangement              | 0                 | 2                     |
| Other                           | 0                 | 3                     |
| **Prior HSCT**                  |                   |                       |
| N                               | 5 (55.5%)         | 9 (90%)               |
| Y                               | 4 (44.4%)         | 1 (10%)               |
