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Authors

Pommert, Lauren
Schafer, Eric S
Malvar, Jemily
et al.

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Decitabine and Vorinostat with FLAG Chemotherapy in Pediatric Relapsed/Refractory AML: Report from the Therapeutic Advances in Childhood Leukemia and Lymphoma (TACL) Consortium

Lauren Pommert, MD[1,2,*], Eric S. Schafer, MD[3,*], Jemily Malvar[4], Nathan Gossai, MD[5], Ellynore Florendo[4], Kirthi Pulakanti[6], Katelyn Heimbruch, PhD[6,7], Cary Stelloh[6], Yueh-Yun Chi, PhD[4,8], Richard Sposto, PhD[4,8], Sridhar Rao, MD, PhD[6,7,9], Thu Van Huynh, MD[10], Patrick Brown, MD[11], Bill H. Chang, MD, PhD[12], Susan I. Colace, MD[13], Michelle L. Hermiston, MD[14], Kenneth Heym, MD[15], Raymond J. Hutchinson, MD[16], Joel A. Kaplan, DO, MPH[17], Rajen Mody, MD[16], Tracey A. O'Brien, MD[18], Andrew E. Place, MD, PhD[19], Peter H. Shaw, MD[20], David S. Ziegler, MD[21,22], Alan Wayne, MD[4,8], Deepa Bhojwani, MD[4,8,^], Michael J. Burke, MD[9,^]

[1]Division of Oncology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[2]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH

[3]Department of Pediatrics, Baylor College of Medicine, Houston, TX

[4]Cancer and Blood Disease Institute, Children's Hospital Los Angeles, Los Angeles, CA

[5]Department of Pediatrics, Center for Cancer and Blood Disorders, Children's Minnesota, Minneapolis, MN

[6]Blood Research Institute, Versiti, Milwaukee, WI

[7]Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA

[8]USC Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, C

Correspondence to: Lauren Pommert, MD MS, Cincinnati Children's Hospital Medical Center, Cancer and Blood Diseases Institute, 3333 Burnet Ave | MLC#7018 | Cincinnati, OH 45229, lauren.pommert@cchmc.org, Phone: 513-803-9083, Fax: 513-636-3549.
* Shared 1st author
^Shared last author

[9]Division of Pediatric Hematology-Oncology, Medical College of Wisconsin, Milwaukee, WI

[10]Department of Pediatrics, Children's Hospital of Orange County, Orange, CA

[11]Division of Pediatric Oncology, Johns Hopkins University, Baltimore, MD

[12]Department of Pediatrics, Oregon Health and Science University, Portland, OR

[13]Department of Pediatrics, Hematology and Oncology, Nationwide Children's Hospital, Columbus, OH

[14]Division of Hematology/Oncology, University of California, San Francisco Benioff Children's Hospital, San Francisco, CA

[15]Department of Pediatrics, Cook Children's Medical Center, Fort Worth, TX

[16]Department of Pediatric and Communicable Diseases Division of Pediatric Hematology and Oncology, University of Michigan Medical School, Ann Arbor, MI

[17]Department of Pediatrics, Carolinas Medical Center/Levine Cancer Institute, Charlotte, NC

[18]Cord & Marrow Transplant Program, Centre for Children's Cancer & Blood Disorders, Sydney Children's Hospital, Sydney, Australia

[19]Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA

[20]Cancer and Blood Disorders Institute, Johns Hopkins All Children's Hospital, St. Petersburg, FL

[21]Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia

[22]School of Women's and Children's Health, University of New South Wales, Sydney, Australia

Abstract

Survival outcomes for relapsed/refractory pediatric acute myeloid leukemia (R/R AML) remain dismal. Epigenetic changes can result in gene expression alterations which are thought to contribute to both leukemogenesis and chemotherapy resistance. We report results from a phase I trial with a dose expansion cohort investigating decitabine and vorinostat in combination with fludarabine, cytarabine, and G-CSF (FLAG) in pediatric patients with R/R AML [NCT02412475]. Thirty-seven patients enrolled with a median age at enrollment of 8.4 (range, 1–20) years. There were no dose limiting toxicities among the enrolled patients, including two patients with Down syndrome. The recommended phase 2 dose of decitabine in combination with vorinostat and FLAG was 10 mg/m^2. The expanded cohort design allowed for an efficacy evaluation and the overall response rate among 35 evaluable patients was 54% (16 complete response (CR) and 3 complete response with incomplete hematologic recovery (CRi)). Ninety percent of responders achieved minimal residual disease (MRD) negativity (<0.1%) by centralized flow cytometry and 84% (n=16) successfully proceeded to hematopoietic stem cell transplant. Two-year overall survival was 75.6% [95%CI: 47.3%, 90.1%] for MRD-negative patients vs. 17.9% [95%CI: 4.4%, 38.8%] for those with residual disease (p<0.001). Twelve subjects (34%) had known epigenetic alterations with 8 (67%) achieving a CR, 7 (88%) of whom were MRD negative. Correlative pharmacodynamics demonstrated biologic activity of decitabine and vorinostat and identified specific gene enrichment signatures in non-responding patients. Overall, this therapy was well-

tolerated, biologically active, and effective in pediatric patients with R/R AML, particularly those with epigenetic alterations.

Keywords

decitabine; vorinostat; relapse; acute myeloid leukemia; epigenetic

Introduction

Despite progress in the treatment of pediatric acute myeloid leukemia (AML), outcomes remain sub-optimal with 5-year overall survival around 70% [1]. The primary treatment challenge is chemotherapy resistance which results in relapse in about 1/3 of patients and <40% survival at 5 years [2–5]. In addition, 10–20% of patients are refractory to upfront therapy with even worse 3 to 5-year survival ranging from 6–19% [3, 6]. Collectively, this illustrates a critical need for development of new strategies to overcome drug resistance and improve survival in children, adolescents, and young adults (AYA) with relapsed/refractory (R/R) AML.

Recent data suggest AML leukemogenesis is highly influenced by aberrant epigenetic events [7–10] with certain subsets of disease governed by specific epigenetic drivers [11, 12]. DNA hypermethylation, loss of histone acetylation, and chromatin modifications play an initiating role in leukemia development and may mediate chemotherapy resistance through silencing of tumor suppressor genes involved in regulating chemosensitivity [13–17]. These alterations can be reversed with epigenetic modifying agents such as decitabine, a DNA methyltransferase inhibitor (DNMTi), and vorinostat, a histone deacetylase inhibitor (HDACi) [18]. DNMTi have been shown to have additive or synergistic effects with HDACi in reactivating epigenetically silenced genes and inducing apoptosis, differentiation, and/or cell growth arrest in cancer cell lines and primary samples [19–22]. Epigenetic agents may be even more effective in subsets of AML which have underlying epigenetic alterations.

Encouraging results have been observed in children with R/R acute leukemias treated with azacitidine, fludarabine, and cytarabine [23] and in adults with AML treated with decitabine and vorinostat [20, 24], however this combination of epigenetic agents has never been reported in children with AML or in combination with chemotherapy. Using epigenetic modifying agents to reverse epigenetic alterations has the potential to restore gene expression, improve chemosensitivity, and result in greater remission rates and improved clinical outcomes. We therefore developed a phase I study for children and AYAs with R/R AML combining two classes of epigenetic modifying agents with a chemotherapy backbone (T2016–003 / NCT02412475). The primary objectives of this study were to determine the maximum tolerated dose (MTD) of decitabine when used in combination with vorinostat, fludarabine, cytarabine, and G-CSF (FLAG), and to evaluate the safety of this combination. Secondary objectives included exploring pharmacodynamic effects of decitabine and vorinostat; overall treatment response; minimal residual disease (MRD) rates; and the safety of delivering this combination in patients with R/R Down syndrome AML (DS-AML).

Methods

The study was conducted by the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Consortium and was reviewed and approved by the institutional review boards of all participating TACL centers. Individual and/or parental informed consent was obtained from all eligible subjects as per local and federal requirements. Eligible patients were 1 to 25 years with AML in >1st relapse or refractory to 2 or more previous induction attempts with measurable disease (>M2 marrow or M1 marrow with MRD defined as >0.1% AML by flow cytometry or molecular testing on 2 serial marrows at least 1-week apart demonstrating stable/rising MRD). Patients who experienced relapse after allogeneic hematopoietic cell transplantation (HSCT) were eligible provided they had no evidence of active graft-versus-host-disease (GvHD) and were at least 60 days post-HSCT. A Karnofsky or Lansky score >50% with adequate renal, hepatic, and cardiac function were required at study entry.

Treatment

Four dose levels of decitabine were to be investigated: dose level 0 (DL0) 5mg/m^2, dose level 1 (DL1) 7.5 mg/m^2, dose level 2 (DL2) 10 mg/m^2, and dose level 3 (DL3) 15 mg/m^2. Patients received decitabine (at assigned dose level) and vorinostat days 1 to 5 followed by FLAG chemotherapy (Supplemental Table 1). Intrathecal chemotherapy (cytarabine or cytarabine, methotrexate, and hydrocortisone) was given up to 72 hours prior to the initial doses of decitabine/vorinostat, with additional weekly intrathecal therapy for central nervous system disease. Patients who achieved a complete/partial response or had stable disease after cycle 1 could receive a second course of therapy which was identical to cycle 1 in schema and doses.

Toxicity Evaluation

Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Dose limiting toxicity (DLT) was defined as any event that was at least possibly attributed to decitabine and was assessed during the first course of treatment only. Non-hematologic DLT was defined as any Grade 3 or 4 non-hematologic toxicity attributed to decitabine with the exception of nausea; alopecia; anorexia; fever/infection; vomiting or diarrhea that returned to Grade <2 within 7 days, mucositis that returned to Grade <2 within 14 days; elevation of transaminases, amylase, lipase, bilirubin, alkaline phosphatase, or GGT that returned to Grade <2 within 14 days; and/or transient electrolyte abnormalities not associated with clinical sequelae. Hematologic DLT was defined as an absence of peripheral blood count recovery [absolute neutrophil count (ANC) >500/μL and platelet count >20,000/μL] within 8 weeks of starting the first dose of protocol therapy, in patients who achieved remission, as documented by marrow aplasia, not marrow infiltration/persistent disease.

Response Evaluation

To evaluate treatment response, a bone marrow evaluation was performed between days 35–42 or when blood counts recovered (ANC >500/μL AND platelet >50,000/μL). A complete response (CR) was defined as attaining an M1 marrow (<5% blasts) with no

evidence of circulating blasts or extramedullary disease (EMD) in addition to recovery of peripheral blood counts (ANC >500/μL and platelet count >50,000/μL). A CR MRD negative (CR MRD-) was defined as a CR with <0.1% MRD by centralized multi-parameter flow cytometry (University of Washington Hematopathology Laboratory, Seattle, WA). CR with incomplete hematologic recovery (CRi) was defined as attaining an M1 marrow with no circulating blasts or EMD and insufficient recovery of ANC (<500/μL) and/or platelets (<50,000/μL). Partial response (PR) was defined as no circulating blasts and achievement of M2 marrow status (5–25% blasts) with recovery of peripheral counts (ANC >500/μL and platelet count >50,000 μL). Stable disease (SD) was designated for patients who did not meet the criteria for PR, CR, or CRi. Progressive disease (PD) was defined as an increase of at least 25% in the absolute number of leukemia cells (circulating blasts or marrow) or development of new EMD sites. Patients were defined as not evaluable (NE) if they did not satisfy the criterion for PD and either did not have a bone marrow evaluation or had a hypocellular marrow.

Statistical Methods

The primary endpoint for dose escalation was the occurrence of a dose limiting toxicity (DLT) during the first course of therapy. Any patient not experiencing a DLT who received less than 80% of the prescribed total dose of any of the systemic anticancer agents for reasons unrelated to decitabine toxicity, or who started subsequent anti-cancer therapy before the required observation times specified in the DLT definition, was considered not evaluable for DLT and was replaced. All other patients who received any portion of treatment were evaluable for DLT. Patients were considered evaluable for treatment response if they received any portion of prescribed protocol therapy and had an evaluable bone marrow sample (Day 35 or when their counts recovered) or had progressive disease by peripheral blood evaluation. The study used a standard 3+3 phase I design for dose determination. If a provisional recommended phase 2 dose (RP2D) was established, accrual could continue until a maximum of 33 DLT- and response-evaluable non-DS patients were enrolled in the primary stratum in order to evaluate secondary endpoints of response and pharmacodynamics analyses. Patients who were not DLT- or response-evaluable were replaced but included in statistical analysis as appropriate. Patients with R/R DS-AML were enrolled in a separate stratum using a 3+3 design, but with the additional restriction that the dose level in DS patients could never exceed the current dose level in the primary stratum. Patient characteristics, responses, and toxicities were summarized with frequencies and percentages. The overall survival (OS) was defined from the start of protocol therapy to death from any cause and summarized by the Kaplan–Meier estimates with standard errors estimated by the Greenwood formula [25]. These analyses were performed using Stata 17 (StataCorp, College Station, TX).

Correlative Studies

Participation in the correlative studies was optional. For patients who participated, peripheral blood and bone marrow were collected prior to the start of therapy and between days 35–42 after completion of therapy and count recovery. Additionally, peripheral blood was collected on study Day 5 post-decitabine/vorinostat therapy and prior to FLAG chemotherapy. Adequate paired pre- and post-epigenetic treatment (Day 0 and Day 5) RNA was isolated

from 11 patients and 4 patients had sufficient material to isolate DNA. DNA samples underwent reduced representation bisulfate sequencing (RRBS) to assess methylation changes before and after epigenetic treatment. RNA-sequencing analysis (RNA-seq) was performed to identify differentially expressed genes between responders and non-responders pre- and post-epigenetic therapy. Gene set enrichment analysis (GSEA) and leading-edge analysis was performed to further classify differentially expressed genes (Supplemental Appendix 1).

Results

Patient Characteristics

Between July 2017 and July 2020, 37 patients with R/R AML were enrolled including 17 (46%) patients who relapsed after a prior bone marrow transplant (4 of whom (11%) were in second relapse), 9 (24%) with disease which was primary refractory two or more courses of upfront therapy, and 2 (5%) with first relapse of DS-AML (Table 1). The median time of follow-up was 21.7 (range, 2.8 – 38.8) months and the median age at enrollment was 8.4 (range, 1–20) years. Thirty-four (92%) patients had >M2 marrow disease at the time of enrollment (M1=2, M2=13, M3=21, 1 without marrow evaluation due to peripheral blasts). Eleven (30%) patients had EMD at study entry (6 CNS, 3 skin, 2 other). Thirteen (35%) patients had genetic alterations that are known to be epigenetically regulated including *KMT2A*r, *CEBPα*, *IDH2*, *NUP98-KDM5A* (Supplemental Table 2).

Toxicity

Of the 37 patients who enrolled on study, 35 were evaluable for DLT. Two patients were not evaluable due to being taken off study secondary to rapidly progressive disease (n=1) and death from progressive disease (n=1) prior to receiving 80% of prescribed systemic therapy (Supplemental Figure 1). No patient experienced DLT (Supplemental Table 3). In cycle 1, the most common Grade 3 and 4 toxicities were hypokalemia (35%), anorexia (17%), elevated AST (17%), hypoxia (17%), hypotension (12%), hyperglycemia (11%), and hypertension (11%) (Supplemental Table 4). Regarding infectious toxicities, 5 (14%) patients experienced Grade 4 sepsis and 6 (17%) experienced lung infections (Grades 3/4). The study was initially suspended after 3 of the first 6 patients who enrolled at decitabine DL3 (15 mg/m^2) developed Grade 4 invasive fungal infections (IFI) (A*spergillus terreus*, *Candida parapsilosis* and *Rhizomucor pusillus)* (Supplemental Table 5). Though DLT criteria were not met, the concern for an increase in IFIs led to a dose reduction of decitabine to DL2 (10 mg/m^2) and requirement (rather than recommendation) of anti-fungal prophylaxis with an echinocandin, extended spectrum azole, or amphotericin agent in all subjects beginning at the start of therapy. After the study was amended and re-opened, an additional 3 (15%) of the subsequent 20 patients enrolled developed systemic fungal infections (*Candida parapsilosis*, *Trichosporon asahii* and *Leptotrichia*) which is consistent with the known rates of fungal infections in pediatric patients with R/R AML treated with FLAG chemotherapy [26–28]. There were no DLTs observed among the 2 patients with DS-AML, who were treated at decitabine DL2.

Response

Thirty-five of the 37 enrolled patients completed protocol therapy and were evaluable for response. The reasons for not being evaluable were death from seizure and asystole secondary to a presumed intracranial event after receiving 1 dose of vorinostat/decitabine (n=1) and a hypocellular marrow (n=1); both patients were excluded from further analysis (Supplemental Figure 1). Twelve (34%) patients received a second course of therapy. Seventy-five percent of responders achieved their best response after course 1 and responses were noted at all decitabine dose levels (Supplemental Table 6). Best responses reported after up to 2 cycles of therapy included a CR/CRi rate of 54% (n=19, 16 CR, 3 CRi) of which 90% (n=17) of responding patients achieved MRD negativity (<0.1%) by centralized flow cytometry (Table 2). Sixteen (94%) of the CR MRD- patients proceeded to HSCT after study completion and 13 of these patients remain alive at last follow-up (Supplemental Table 2). Of the remaining 16 patients, 1 had PR (3%), 6 had SD (17%), and 9 had PD (26%) (Table 2). Two-year OS for all evaluable patients was 46.9% [95% CI 29.6%, 62.4%] (Figure 1A). When evaluated based on MRD status, those patients who achieved CR MRD- by the end of course 2 (n=17) had a 2-year OS of 75.6% [47.3%, 90.1%] versus 17.9% [4.4%, 38.8%] for those with residual disease (n=18) (p<0.001) (Figure 1B). None of the patients with residual disease were alive at 3-years (Figure 1B).

Of the evaluable patients, 12 (34%) had genetic alterations that are known to be epigenetically regulated (Supplemental Table 2). Among these patients, the CR/CRi rate was 67% (n=8) and 7 (88%) of the responders also achieved MRD negativity (<0.1%) (Table 2, Supplemental Table 7). The only cytogenetic group that did not have a favorable treatment response was FLT3-ITD (n=5), none of whom achieved a CR (Supplemental Table 2). The responses of patients with DS-AML were included with the overall cohort. Within this group, 1 patient reported PD and the other achieved CR MRD- after 1 cycle of therapy, proceeded to HSCT, and remains alive at last follow up (Supplemental Table 2). Neither of these patients had known epigenetic alterations. Of the patients with primary refractory disease (n=9), 4 (44%) reported a CR, 3 of whom were MRD negative. Of the patients who relapsed after prior HSCT (n=15), 6 (40%) achieved CR and 5 were MRD negative.

Correlative Studies

Comparison of DNA methylation changes—We used RRBS to evaluate DNA methylation on a genome-wide scale, comparing peripheral blood samples from pre- (Day 0) and post- (Day 5) epigenetic therapy. Four patients (8 total samples) had sufficient material for paired DNA sample analysis. In all samples tested, we observed significant loss of global DNA methylation across CpG islands (CpGi) after 5 days of treatment with decitabine compared to pre-study samples, signifying a clear pharmacodynamic effect (Supplemental Figure 2A, Supplemental Figure 2B, Wilcoxon test p<2.2e-16). The mean percent methylation loss for all patients was 40% (range, 37% to 47%, n=4). Overall, we observed interpatient variability in extent of demethylation and no correlation between the extent of demethylation and clinical response to treatment, although this result was limited by the small numbers. As all patients with available samples were treated at decitabine dose level 2 (10 mg/m^2), we were unable to investigate dose dependent changes in methylation.

We next evaluated the specific DNA segments that underwent methylation changes in response to treatment and identified 55 shared differentially methylated CpG regions (DMRs) among all 4 patients who had a significant change in methylation >25% (q<0.01) on Day 5 compared to Day 0. Of these 55 DMRs, 16 (29%) were hypermethylated and 39 (71%) were hypomethylated post-epigenetic treatment. Next, we evaluated the nearest genes to the hypomethylated DMRs to identify those potentially impacted by decitabine/vorinostat and nearby genes that were shared across all patients (*NTMT1, USF1, ENPP2, NLRP3, LRRN2*) (Supplemental Table 8). These genes are known to be involved in mitosis and DNA damage; gene transcription; stimulation of cell proliferation and chemotaxis; regulation of inflammation and apoptosis; and cell-adhesion and signal transduction, respectively.

Identifying gene expression changes and biologic pathways affected by epigenetic therapy—Using RNA-seq analysis, differentially expressed genes were compared between responders and non-responders on Day 0 (n=11) and Day 5 (n=10) with 240 differentially expressed genes identified (p=<0.05) (Supplemental Figures 3 and 4). Six genes were upregulated in responders and downregulated in non-responders and the remaining 234 genes were down regulated in responders and upregulated in non-responders. We focused specifically on 90 genes that had decreased expression in responders on Day 0 and increased expression on Day 5 to represent genes that potentially underwent epigenetic modification related to therapy. The unsupervised heatmap of expression of these 90 genes for all patients at Day 0 and corresponding Day 5 showed a heterogenous response across samples (Supplemental Figure 5). However, one general trend identified responders having a global quantitatively higher increase in expression of this subset of genes, which was attenuated in the non-responders. Gene ontology (GO) enrichment analysis of these differentially expressed genes revealed a number of genes involved in relevant biological processes including: stem cell regulation (*DPPA4, FGF2, HMGA2, SFRP1*), transcriptional regulation (*E2F8, HMGA2, IRF6, MEIS2*), epigenetics (*DPPA4*), cell survival and tissue repair (*E2F8, FGF2, FGF13, NEO1, PDGFRA, SDC1, TEK*), apoptosis (*SULF1, TPX2*), tumor growth (*DPPA4, FGF2, FGF13, HPSE2, NEO1*), regulation of cell cycle progression and DNA replication (*CDC6, CLSPN, E2F8, HMGA2, PARD3B, SFRP1, SKA1, SPC25, TPX2*), and DNA damage repair (*POLQ, RAD51AP1, SCARA3, TPX2*). A full list of all 90 genes with increased expression at Day 5 compared to Day 0 can be found in Supplemental Table 9 and those with involvement in relevant biological pathways in Supplemental Table 10. Additionally, several genes with increased expression are implicated in AML as potential risk-loci based upon Genome-Wide Association Studies (GWAs) including: *CCDC113, CDC6, DMGDH, FAM171A2, HPSE2, ITGBL1, LINC01234, MYO3B, PARD3B, PDE8, PDGFRA, PSAT1, ROBO1, SCARA3* (Supplemental Table 11).

Next, we utilized GSEA hallmark gene databases and compared responders to non-responders at Days 0 and 5 to identify signatures that could potentially predict response to therapy. We discovered that non-responding patients demonstrated Day 0 enrichment of genes involved in the G2/M checkpoint progression (NES 1.89, p value 0) and genes encoding cell cycle related targets of E2F transcription factors (NES 1.87, p value 0; Figure 2). Leading edge analysis identified 52 genes shared among non-responding patients within these pathways with some of the more interesting genes being: *AURKB, BRCA2, PDS5B,*

RAD21, SMC1A, SRF1, SRF2, STAG1 (Supplemental Table 12). At Day 5, non-responding patients demonstrated enrichment in genes involved in the P53 pathway (NES 1.62, p value 0), apoptosis (NES 1.50, p value 0), TNFA signaling via NFKB (NES 1.94, p value 0), interferon gamma response (NES 1.57, p value 0), interferon alpha response (NES 1.53, p value 0.001), IL-6 JAK STAT3 signaling (NES 1.66, p value 0), and reactive oxygen species pathway (NES 1.63, p value 0.001; Figure 2).

Discussion

R/R AML remains one of the most challenging diseases to effectively treat in pediatric oncology [4]. The dismal prognosis for these patients is most often the result of chemotherapy resistance. Identifying ways to improve treatment response by targeting specific mutations, overcoming chemotherapy resistance, and minimizing toxicities are required so that more patients can undergo potentially curative consolidation with HSCT. Although there have been clinical trials using decitabine and vorinostat in combination in elderly adults with AML [20, 24] and in children with ALL [29, 30], this combination of epigenetic agents has never been given to pediatric patients with AML in conjunction with chemotherapy. Our study goal was to epigenetically modify leukemia cells using a 5-day epigenetic window to improve sensitivity to standard chemotherapeutic agents. Overall, we identified decitabine (10 mg/m^2) in combination with vorinostat and followed by FLAG chemotherapy to be well-tolerated and effective in pediatric patients with R/R AML. Despite our population of patients being heavily pre-treated, the side effect profile we reported was consistent with other intensive AML regimens with 14% experiencing Grade 4 sepsis and 17% fungal infections.

The response rates after up to two cycles of therapy in this heavily pre-treated AML population were promising with a CR/CRi rate of 54%. It is notable that 90% of the responding patients achieved MRD negativity (<0.1%) despite high disease burden in 95% of patients (>M2) at the time of study enrollment. Thirty-four percent of patients (n=12) tolerated their 1st cycle of therapy and proceeded to a second cycle, although 75% of patients had their best response with their first cycle. Eight (67%) of the patients who received a second cycle either maintained or achieved an MRD negative response. Forty-four percent of the primary refractory patients achieved a CR and 75% of them became MRD negative. Ninety-four percent of the responding CR MRD- patients went on to receive successful HSCT after therapy completion (Supplemental Table 2), reporting 2-year OS of 75.6% [95% CI 47.3%, 90.1%] (Figure 1B). Although the overall numbers are relatively small and the study was not powered to detect differences among cytogenetic subgroups, a higher percentage of clinical responses was observed in patients with known epigenetic alterations (i.e. *KMT2A*r, *CEBP*α, *IDH2, NUP98-KDM5A)* with CR/CRi rates of 67% (n=8; Table 2). Seven (88%) of these patients became MRD negative, all went on to receive HSCT, and 4 remain alive at last follow-up (Supplemental Table 2). The study enrolled two patients with DS-AML who tolerated the study therapy without apparent increased toxicity or DLT. Their responses were included in the overall cohort with 1 reporting PD and the other CR MRD- who also remains a survivor after HSCT (Supplemental Table 2). Importantly, to our knowledge, this represents the first phase 1 clinical trial in pediatric

AML to include patients with Down syndrome, a population that has previously been excluded due to concerns for increased risk of treatment-related toxicity.

This is the first clinical trial of a DNMTi and HDACi in which RRBS and RNA-seq were used to assess epigenetic pharmacodynamics in pediatric patients with AML. We performed correlative analyses including DNA methylation using RRBS in matched Day 0 and Day 5 peripheral blood samples from 4 patients (3 responders and 1 non-responder). Our results clearly demonstrate biological activity of these epigenetic agents in the subset of patients for whom samples were available as evidenced by significant CpGi hypomethylation post-decitabine treatment. Interestingly, one of the patients who carried a known epigenetically-driven lesion (*NUP98-KDM5A*) exhibited the highest rate of demethylation (47% decrease in CpGi methylation) post-decitabine treatment (Supplemental Figure 2). Due to our small sample size, our ability to assess the impact of methylation on clinical remission rates or detect differences between dose levels or cytogenetic subgroups was limited.

Among responding and non-responding patients, there were differences noted in RNA-seq expression profiles on Day 0 and Day 5. Two-hundred-forty differentially expressed genes by RNA-seq were discovered among responders and non-responders. Genes that had decreased expression in responders on Day 0 and increased expression in responders on Day 5 (n=90) were used to represent genes whose expression was potentially impacted by epigenetic therapy. These genes were mapped to biologically relevant networks including cell cycle and DNA replication, transcriptional regulation, epigenetics, apoptosis, tumor growth, and DNA damage repair (Supplemental Tables 8 and 9). Interestingly, the GSEA results from RNA-seq data clearly demonstrated specific gene signatures associated with non-responding patients on Day 0 and Day 5. Genes involved in the G2/M checkpoint progression and genes encoding cell cycle related targets of E2F transcription factors were enriched in non-responders at Day 0. Additionally, non-responders also demonstrated enhancement of genes enriched in inflammatory pathways, apoptosis, and reactive oxygen pathways on Day 5. While further validation is needed, these results suggest that specific gene enrichment signatures from peripheral blood may have the ability to differentiate responders from non-responders prior to the start of therapy. In addition, given the enrichment of apoptosis genes in non-responders, this may indicate non-responders could benefit from apoptosis-promoting agents such as venetoclax. As such, a successor to this trial is currently in development through the TACL Consortium with the addition of venetoclax to epigenetic agents on a FLAG chemotherapy backbone.

In summary, treatment with epigenetic medications followed by chemotherapy in this trial demonstrated encouraging response rates with high rates of MRD negativity in this heavily pre-treated population compared to other R/R AML trials [23, 31–33]. This regimen had an acceptable toxicity profile and should be considered as potential salvage therapy of R/R pediatric AML patients, including those with Down syndrome, and particularly those with known epigenetic alterations. The limitations of this study include the heterogeneous patient population in regard to previous therapy and cytomolecular genetics. Due to the small number of patients who had adequate samples for correlative studies, we were unable to correlate genome-wide methylation effects of treatment with clinical responses or to integrate the RRBS and RNA-seq data; however, this should be explored in future studies.

Additionally, these correlative studies were performed on peripheral blood which had a low level of circulating leukemia cells. Nevertheless, this is the first report of exploratory GSEA analysis pre- and post-epigenetic therapy in matched samples from pediatric patients with R/R AML treated on a clinical trial. Results clearly demonstrate a pharmacodynamic effect of these epigenetic medications with specific GSEA signatures pre- and post-epigenetic therapy that can differentiate responders from non-responders. These results suggest that epigenetic therapy prior to conventional chemotherapy can be a fruitful approach in improving response for subsets of children with R/R AML. Further, identification of patients more likely to respond to this approach might be able to be identified at diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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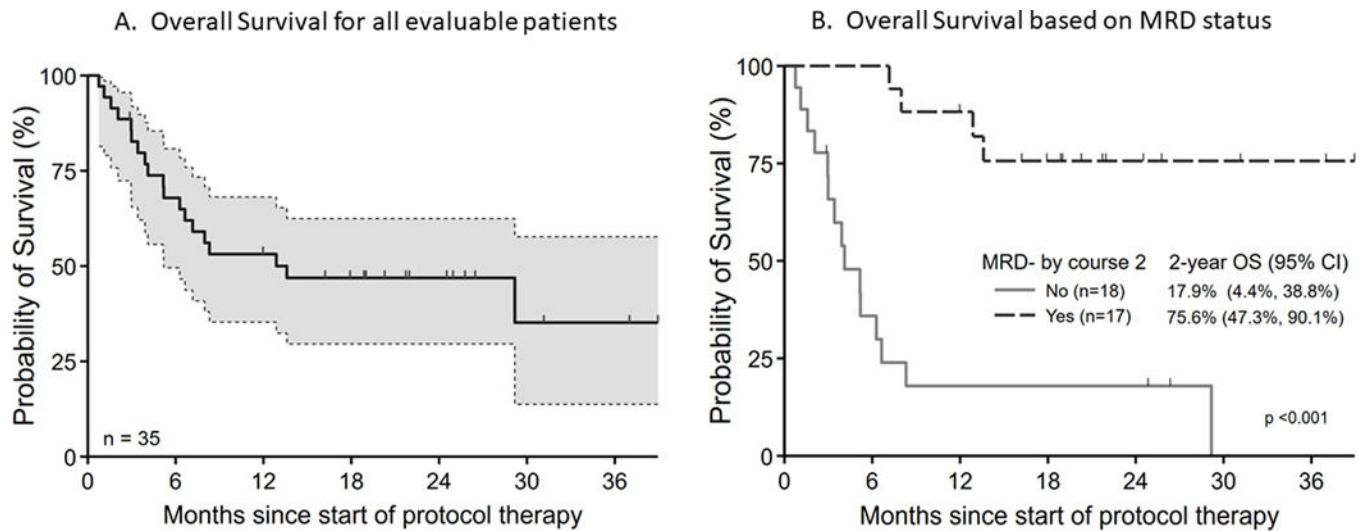
A. Overall Survival for all evaluable patients

B. Overall Survival based on MRD status

Figure 1:

A) Two- year overall survival for all evaluable patients was 46.9% [95% CI 29.6%, 62.4%]

B) Two-year overall survival based on MRD status demonstrated patients who achieved CR MRD- by the end of course 2 (n=17) had a 2-year OS of 75.6% [47.3%, 90.1%] versus 17.9% [4.4%, 38.8%] for those with residual disease (n=18) (p<0.001).
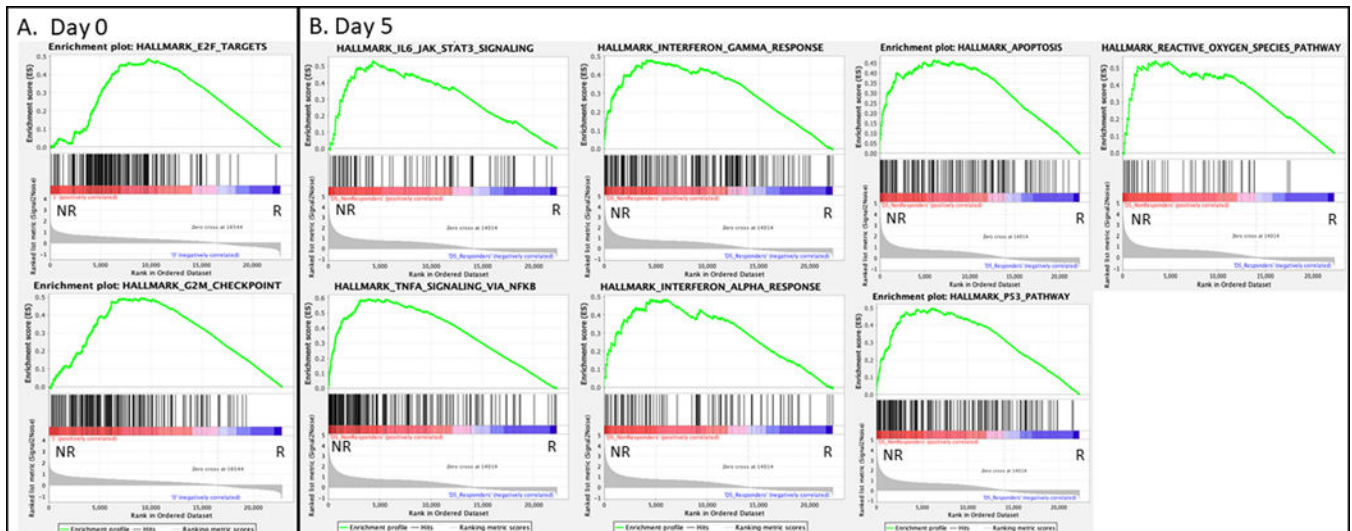
Figure 2:

GSEA signatures for non-responders A) Demonstrates on Day 0 non-responders were enriched in genes involved in the G2/M checkpoint progression (NES 1.89, p value 0) and genes encoding cell cycle related targets of E2F transcription factors (NES 1.87, p value 0). B) Demonstrates on Day 5 non-responders were enriched in genes involved in TNFA signaling via NFKB (NES 1.94, p value 0), IL-6 JAK STAT3 signaling (NES 1.66, p value 0), reactive oxygen species pathway (NES 1.63, p value 0.001), P53 pathway (NES 1.62, p value 0), interferon gamma response (NES 1.57, p value 0), interferon alpha response (NES 1.53, p value 0.001), and apoptosis (NES 1.50, p value 0). R, responder; NR, non-responder

Table 1.

Patient Characteristics

Patient Characteristics	Total (%)
Total enrolled	37
Sex	
Male	25 (67%)
Female	12 (32%)
Age at enrollment (years)	
Median (range)	8.4 (1.0, 20.5)
Race	
White	23 (62%)
Black / African American	4 (10%)
Not Reported	10 (27%)
Ethnicity	
Latino	7 (18.9%)
Not-Latino	26 (70%)
Not Reported	4 (11%)
Down Syndrome	
No	35 (95%)
Yes	2 (5%)
CNS Status	
Positive	6 (16%)
Negative	30 (81%)
Not Evaluated	1 (3%)
Non-CNS Extramedullary Disease	
Skin	3 (8%)
Other	2 (5%)
None	32 (86%)
Prior HSCT	
Yes	17 (46%)
No	20 (54%)
Relapse # at Enrollment	
1st Relapse	24 (65%)
2nd Relapse	4 (11%)
Primary Refractory	9 (24%)
Marrow Disease Burden at Enrollment	
M1 (<5%)	2 (5%)
M2 (>5% to <25%)	13 (35%)
M3 (>25%)	21 (57%)

Patient Characteristics	Total (%)
Marrow not evaluated [†]	1 (3%)
AML Genetics [*]	
Favorable	7 (19%)
Neutral	10 (27%)
Unfavorable	20 (54%)
Epigenetic Lesion (*KMT2Ar, CEPBa, IDH2, NUP98-KDM5A*)	
Present	12 (32%)
Absent	25 (68%)

CNS, central nervous system; HSCT, hematopoietic stem cell transplant

[†]
Marrow not evaluated due to presence of peripheral blasts

[*]
Genetics: Favorable defined as CBF, inv(16), NPM1, CEBPa; Unfavorable defined as FLT3, Monosomy 7, 5q-, KMT2Ar, NUP98 fusions, t(6;9); Neutral cytogenetics defined as neither favorable or unfavorable

Table 2

Summary of Best Response by Decitabine Dose Level and Epigenetic Alterations

Summary of Best Response by Decitabine Dose Level				
	Dose Level 1 7.5 mg/m² (n=3)	**Dose Level 2 10 mg/m² (n=21)**	**Dose Level 3 15 mg/m² (n=11)**	**All Dose Levels (n=35)**
CR/CRi *MRD negative*	1 (33%) *1 (100%)*	14 (67%) *13 (93%)*	4 (36%) *3 (75%)*	19 (54%) *17 (90%)*
PR	-	-	1 (9%)	1 (3%)
SD	1 (33%)	2 (10%)	3 (27%)	6 (17%)
PD	1 (33%)	5 (24%)	3 (27%)	9 (26%)
Summary of Best Response by Epigenetic Alterations				
	Present (n=12)	**Absent (n=23)**	**Total (n=35)**	
CR/CRi *MRD negative*	8 (67%) *7 (88%)*	11 (48%) *10 (91%)*	19 (54%) *17 (90%)*	
PR	-	1 (4%)	1 (3%)	
SD	1 (8%)	5 (22%)	6 (17%)	
PD	3 (25%)	6 (26%)	9 (26%)	

CR, complete response; CRi, complete response with incomplete hematologic recovery; MRD, minimal residual disease; PD, progressive disease; PR, partial response; SD, stable disease