



Published in final edited form as:

J Pediatr Hematol Oncol. 2017 October ; 39(7): 560–564. doi:10.1097/MPH.0000000000000868.

Epigenetic combination therapy for children with secondary myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) and concurrent solid tumor relapse

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Abstract

Secondary myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) is a rare but devastating complication of solid tumor treatment involving high dose topoisomerase II inhibitor and alkylator chemotherapy. For relapsed or elderly MDS and AML patients ineligible for hematopoietic stem cell transplantation (HSCT), epigenetic therapies, including DNA methyltransferase inhibitors and histone deacetylase inhibitors, have been utilized as palliative therapy, offering a well-tolerated approach to disease stabilization, prolonged survival, and quality of life. Literature on the use of epigenetic therapies for both primary and relapsed disease is scarce in the pediatric population. Here, we report two pediatric patients with secondary AML and MDS, respectively, due to prior therapy for metastatic solid tumors. Both patients were ineligible for HSCT due to concurrent solid tumor relapse, but were treated with the epigenetic combination therapy, decitabine and vorinostat, and achieved stabilization of marrow disease, outpatient palliation, and family-reported reasonable quality of life.

Introduction

Secondary myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are potential life-threatening complications of high dose topoisomerase II inhibitor and alkylator therapy for solid tumors in children [1–3]. For adult patients ineligible for hematopoietic stem cell transplantation (HSCT) due to age and/or prohibitive co-morbidities, epigenetic-modifying agents offer a well-tolerated and effective mode of palliative therapy with prolongation of survival and quality of life [4]. There is also emerging data that combination epigenetic therapy can stabilize certain solid tumors [5, 6]. Therefore, combination

epigenetic therapy holds appeal for patients ineligible for HSCT due to concurrent relapse of a solid tumor.

The nucleoside analog, decitabine, is a DNA methyltransferase (DNMT) inhibitor which has been shown to have anti-neoplastic activity by demethylating aberrantly hypermethylated promoters, allowing for reactivation of silenced tumor suppressor genes and regulatory microRNA [4]. Decitabine (Dacogen, Eisai Inc., New Jersey, NJ, USA; 5-aza-2'-deoxycytidine) and its related azanucleoside, azacitidine, have become the standard of care for older adults with MDS/AML unable to tolerate intensive chemotherapy and HSCT, offering prolongation of survival with improvement in hematopoiesis and durable transfusion-independency [7].

Histone deacetylase (HDAC) inhibition also induces expression of previously silenced genes by causing an open chromatin structure allowing for gene transcription [8]. In preclinical studies, HDAC inhibitors stimulated growth arrest and activated apoptotic pathways [9, 10]. Specifically, HDAC inhibitors have been shown to affect a host of cellular targets leading to antitumor activity in myeloid malignancies [4]. Vorinostat (Zolinza, Merck, Whitehouse Station, NJ; suberoylanilide hydroxamic acid), a small molecule oral inhibitor of class I and II HDAC enzymes has been widely incorporated into clinical trials for adult malignancies and was FDA approved for treatment of cutaneous T cell lymphoma. A phase I study of Vorinostat for the treatment of adults with advanced leukemia and MDS showed promising clinical activity particularly in patients with AML [11]. Vorinostat is now undergoing early phase pediatric clinical trials for a wide variety of pediatric malignancies [12].

Preclinical studies have demonstrated synergy between DNMT and HDAC inhibitors against a wide range of cancers including leukemias [8, 13, 14]. Based on this, a phase I study was designed evaluating the combination of vorinostat and decitabine for older adults with untreated MDS/AML, refractory MDS/AML, and intermediate to high grade MDS, which showed that this regimen was safe and well-tolerated with promising clinical activity particularly in the untreated AML group [4]. A number of pediatric trials have been conducted examining the use of an HDAC or DNMT inhibitor alone or in combination with cytotoxic therapy for relapsed/refractory cancers [12, 15, 16]. However, patients with both a relapse of their solid tumor and secondary MDS/AML are generally not eligible for clinical trials. Here we report two patients with concurrent solid tumor relapse and secondary AML and MDS, respectively, who were ineligible for clinical trials and HSCT. Both patients were treated with the MDS/AML-directed palliative therapeutic regimen, decitabine and vorinostat, achieving stabilization of marrow disease and allowing for outpatient palliation with family-reported reasonable quality of life.

Case Series

Patient 1 is a fifteen year old girl who initially presented with an enlarging mass of the right mid-thigh, abdominal pain and cough. Imaging revealed a right retrocrural mass, multiple mediastinal lymph nodes, and a retroperitoneal mass compressing the aorta. A biopsy showed alveolar rhabdomyosarcoma and PET scan revealed widely metastatic disease including bony involvement. FISH analysis for FOXO1A (FKHR; 13q14.1) rearrangement

was positive. Bilateral bone marrow biopsy and aspirates were negative with normal karyotype. She was enrolled on the Children's Oncology Group (COG) High Risk Rhabdomyosarcoma clinical trial (ARST08P1) and was randomized to receive Cixutumumab in combination with intensive multi-agent interval compressed therapy, including vincristine, irinotecan, doxorubicin (cumulative dose 375 mg/m²), dactinomycin, cyclophosphamide (cumulative dose 9.6 g/m²), ifosfamide (cumulative dose 45 g/m²), and etoposide (cumulative dose 2.5g/m²). She also underwent radiation therapy to her abdomen and right thigh lesion. She achieved a complete remission by six months on therapy.

At six months off therapy (18 months from diagnosis) she was diagnosed with relapsed disease on surveillance imaging with PET-avid lesions of the hilum and lungs, retroperitoneum, and bilateral breasts. Bilateral bone marrow biopsy and aspirates were positive for metastatic rhabdomyosarcoma and also revealed myeloid hyperplasia with dyspoiesis of the myeloid and erythroid series by morphology. Karyotype showed a t(2;11) (q31;p15) mutation and deletion 7q. FISH analysis for FOXO1A (FKHR; 13q14.1) rearrangement was negative. Her CBC was normal and there were no peripheral blasts. Treatment for relapsed rhabdomyosarcoma was initiated as per the COG protocol ARST0921 with Vinorelbine and Cyclophosphamide (cumulative dose 3.6 g/m²) After 3 cycles of therapy, myeloblasts were noted on peripheral smear and peripheral blood flow cytometry confirmed acute myeloid leukemia CD33+, CD34+, HLA-DR+, CD7dim, CD79a dim, CD13-, CD117-, CD19-, CD10-, CD3-, and TdT-. She received 5 additional cycles of Vinorelbine and Cyclophosphamide (cumulative dose 6 g/m²), maintaining stable disease of her rhabdomyosarcoma during which time her AML remained stable (peripheral blast count at 1–2%). However, during the 5th cycle, her white blood cell count began rising to a maximum of 139K/ul with 89% peripheral blasts.

Due to her relapsed rhabdomyosarcoma, she was determined to be ineligible for HSCT and she initiated palliative therapy with decitabine 20mg/m²/day for 5 days per 4 week cycle [17] and vorinostat 200mg twice daily for 14 days per cycle together with hydroxyurea. During the first two cycles of therapy she continued to have profound leukocytosis (range 72–255 K/ul) with predominance of blasts. However, by the third cycle of therapy she had normalization of her white blood cell (WBC) count (range 0.9–3.9 K/ul) and a decrease in blast percentage (range 2–36%) and hydroxyurea was discontinued. Her WBC count remained stable for 4 cycles of therapy during which time she experienced grade 3 myelosuppression with transfusion dependency, but no other toxicities. She remained outpatient throughout this time. However, at the start of her fifth cycle of therapy the WBC count increased to >200K/ul. She developed progressive pain and extremity numbness. She received a sixth cycle of therapy but then developed altered mental status and respiratory distress. Chemotherapy was discontinued and she died several days later.

Patient 2 is a 13 year old girl who was initially diagnosed at the age of 7 with stage 4 neuroblastoma (NMYC non-amplified). She presented with an abdominal mass, mediastinal lymphadenopathy, widely metastatic bone and bone marrow disease. Induction therapy was initiated as per the COG protocol A3973 with 5 cycles of chemotherapy including cyclophosphamide (cumulative dose 12.6 g/m²), doxorubicin (cumulative dose 225mg/m²), etoposide (cumulative dose 1.2 g/m²), cisplatin, and vincristine[18]. Following cycle 5 she

had surgical resection of the primary abdominal tumor and all accessible metastatic disease in the mediastinum. She received Cycle 6 of therapy with high-dose cyclophosphamide, topotecan, and vincristine [19]. Consolidation treatment consisted of myelo-ablative chemotherapy with etoposide, carboplatin, and melphalan, followed by stem cell rescue. Disease evaluation following consolidation showed remission.

She then received 9 monthly cycles of 3F8 antibody therapy as well as cis-retinoic acid and underwent radiation therapy (XRT) to the sites of initial soft tissue disease. Following the 9th cycle of 3F8, routine imaging studies showed recurrence of disease in mediastinal lymph nodes (relapse #1). She was treated with another cycle of high-dose cyclophosphamide, topotecan, and vincristine [19]. She underwent focal XRT (2100cGy) to the mediastinum, followed by 8 cycles of therapy with temozolomide and irinotecan [20]. Imaging studies showed second remission. She was placed on maintenance therapy for 11 cycles with daily celecoxib and lenalidomide. Routine imaging studies during cycle 11 showed relapse again in mediastinal lymph nodes and the abdomen (relapse #2). She received a third cycle of high-dose cyclophosphamide, topotecan, and vincristine. She had surgical resection of the abdominal disease, followed by one cycle of intermediate dose cyclophosphamide, topotecan, and vincristine and one cycle of single agent irinotecan, followed by focal XRT (2100cGy) to the site of abdominal relapse. She then received 2 cycles of cis-retinoic acid. The patient was enrolled on the COG protocol ADVL0921 and received one cycle of MLN8237, an Aurora A kinase inhibitor, which was discontinued due to prolonged neutropenia. She was then enrolled on a phase I trial of EZN-2208 (PEGylated SN-38) and continued on this treatment for one year with stable disease.

However, on a routine extent of disease evaluation, 5.5 years from initial diagnosis, she was noted to have both disseminated neuroblastoma (now with multifocal bone and bone marrow as well as soft tissue disease) and myelodysplasia characterized by 5q deletion and monosomy 7 on karyotyping and FISH in 85% and 81% of tested cells respectively. Cumulative doses of doxorubicin, cyclophosphamide and etoposide were 225mg/m², 34.4g/m², and 2.55g/m² respectively. As the patient was deemed ineligible for HSCT due to her relapsed neuroblastoma, she initiated MDS-directed palliative therapy with decitabine (20 mg/m² per day ×5 days/cycle) and vorinostat (100 mg/m² per day for 14 days/cycle) in a 28 day cycle. She was also started on oral etoposide at 50 mg/m²/day concurrently for neuroblastoma-directed palliative therapy. Disease evaluation by CT scan showed response to therapy with only soft tissue disease in the mediastinum. She developed prolonged pancytopenia so vorinostat was omitted from cycle 2 and etoposide discontinued after cycle 2. She received an autologous stem cell infusion 2 days after the second cycle. The stem cells were from the original collection during induction therapy and normal cytogenetics were confirmed prior to infusion. She had neutrophil recovery by day + 11 with an absolute neutrophil count (ANC) of > 1000, RBC transfusion independency by day + 13, and platelet transfusion independency by day + 23, with platelets recovering to greater than 75,000 by day + 45. Bone marrow examination performed 6 weeks after her stem cell infusion was negative for 5q- or monosomy 7. Morphology showed mild hypocellularity with delayed maturation and flow cytometry was normal. Decitabine and vorinostat therapy was resumed 2 months after stem cell infusion for cycle 3. Following cycle 3, FISH testing on bone marrow revealed 14% of cells had both 5q deletion and monosomy 7. Disease surveillance

studies showed further progression of neuroblastoma in the neck and mediastinum and with widespread bony disease. She was treated with a cycle of decitabine (dose reduced to 10 mg/m² per day ×5 days) plus ICE (dose-reduced ifosfamide, carboplatin, etoposide), followed by another stem cell infusion. Unfortunately, her neuroblastoma continued to progress rapidly. She received palliative radiation therapy to the cervical and thoracic spine bones for pain control after which the patient and family decided to discontinue all cancer-directed therapy and she died soon after, 11 months from discovery of the MDS. Her MDS never converted to AML, though there was progressive neuroblastoma. During this period, she experienced grade 3 myelosuppression with requirements for platelet transfusions 1–2 times weekly for 2–3 weeks of each cycle and RBC transfusions about every 2 weeks (although no RBC transfusions needed during cycle 3 of decitabine and vorinostat). She had 4 admissions for fever and neutropenia without a source, but remained outpatient for 6 months of the last 11 months of life and had no other toxicities attributed to the epigenetic combination therapy. The family reported excellent quality of life during this time as the patient travelled extensively, participated in normal activities and attended school part time..

Discussion

Here we report two pediatric patients who developed secondary MDS/AML in the context of their relapsed metastatic solid tumors. Both patients received MDS/AML-directed palliative therapy with the epigenetic combination, vorinostat and decitabine, achieving stabilization of marrow disease and reasonable quality of life. Patient 1 with secondary AML experienced a significant response to therapy lasting 4 months but ultimately succumbed to refractory AML and likely progressive solid tumor disease; Patient 2 with secondary MDS achieved stabilization of MDS without conversion to AML but succumbed to progressive neuroblastoma 11 months after MDS diagnosis.

Treatment-related MDS/AML is a rare but catastrophic late effect of chemotherapy for advanced solid tumors. The DNA topoisomerase II inhibitors, particularly etoposide and doxorubicin, and the alkylators have been implicated as the greatest risk agents with direct correlation of the cumulative dose to relative risk. In a case control study of French pediatric patients treated for solid tumors, Le Deley, et al. found that those who received 1.2–6g/m² of etoposide or more than 170mg/m² of anthracyclines had a 7-fold higher risk of developing secondary leukemia as compared to those who received lower doses or none at all (reference group). Furthermore, the risk of leukemia in patients who received more than 6g/m² of etoposide was multiplied by 197 as compared to the reference group. They did not, however, find an increased risk of leukemia in patients exposed to alkylators [1]. In contrast, Bhatia, et al. found, in a retrospective study of 578 children treated for Ewing sarcoma on the Children's Oncology Group protocol INT-0091, that an increase in ifosfamide exposure from 90 to 140g/m², cyclophosphamide from 9.6 to 17.6g/m², and doxorubicin from 375 to 450 g/m² with no change in etoposide cumulative dose of 5g/m² led to a 16-fold increased risk of treatment-related MDS/AML (cumulative incidence of 11% at 5 years) in patients treated on regimen C receiving the higher doses of chemotherapy [21]. Similarly, Kushner, et al. reported that the 5-year cumulative incidence of treatment-related AML/MDS in a cohort of 108 pediatric patients treated at MSKCC for high risk neuroblastoma with 6–8 cycles of alkylator-heavy combination chemotherapy involving cyclophosphamide (16.8–21

g/m²), doxorubicin (300–375 mg/m²), and etoposide (1.8 g/m²) was 7.1% as compared to 0% in those who received 5 cycles [22]. Both patients reported here required intensive multi-modal chemotherapy for high risk metastatic solid tumors, involving topoisomerase II inhibitor therapy at cumulative dosing of 2.5g/m² and alkylator therapy including cyclophosphamide at cumulative dosing of 13.2 g/m² and 34.4 g/m² (patients 1 and 2, respectively) and doxorubicin at cumulative dosing of 375 mg/m² and 225 mg/m² (patients 1 and 2, respectively), rendering them at risk for secondary MDS/AML.

Due to concurrent solid tumor relapse, neither patient was deemed eligible for phase I/II clinical trials or HSCT and palliative therapy options had to be considered. The DNMT inhibitor, decitabine, has been FDA-approved for treatment of MDS/AML in adults and has become standard of care for upfront therapy in older adults who cannot tolerate more intensive therapy. Mechanistically, decitabine has been shown to correct aberrant methylation patterns in DNA leading to reactivation of silenced tumor suppressor genes [23]. Preclinical studies have suggested that HDAC inhibitors may synergize the epigenetic modulation of hypomethylating agents, improving outcomes in MDS/AML [8, 13, 14]. Based on these discoveries, Kirschbaum, et al. conducted a Phase I clinical trial of vorinostat in combination with decitabine for adult patients with MDS or AML and found that the combination, both in concurrent and sequential scheduling, was safe and well-tolerated and that the concurrent schedule offered promising clinical activity [4]. A number of other non-randomized Phase I/II studies combining an HDAC inhibitor with a hypomethylating agent have similarly shown promising anti-leukemia/MDS activity [24, 25]. However, randomized clinical trials have yet to support these findings. For example, Issa, et al. compared the use of decitabine alone to decitabine with the HDAC inhibitor, valproic acid, in a Phase II randomized clinical trial and did not find a statistically significant difference in outcomes for MDS/AML [26]. Similarly, the US Leukemia Intergroup Trial E1905 did not demonstrate a statistically significant difference in survival with the addition of the HDAC inhibitor, entinostat, to azacitidine for MDS/AML [27]. It has been postulated that optimal scheduling of the drug combinations and strength of the HDAC inhibitors available for these studies may have impacted study results and further studies are warranted to examine alternative schedules and more potent HDAC inhibitors. Vorinostat is considered to be a more potent HDAC I and II inhibitor than those used in these studies and no phase II randomized clinical trial results combining decitabine with vorinostat for MDS/AML have yet been reported.

There is a growing interest in the use of epigenetic therapies for treatment of cancer in children, but data is scarce for use in pediatric MDS/AML. One retrospective study showed that low-dose azacitidine for children and adolescents with MDS was safe and effective as a non-toxic option to prolong life in the palliative context [28]. Furthermore, a pilot study of 13 pediatric patients investigating the use of vorinostat and decitabine followed by a standard cytotoxic re-induction chemotherapy regimen for relapsed/refractory B-acute lymphoblastic leukemia (B-ALL) showed that the regimen was tolerable and demonstrated clinical benefit with an overall response rate of 46.2% (complete and partial response) [15]. However, the subsequent expanded pilot study investigating an extended schedule of decitabine and vorinostat on a similar chemotherapy backbone for relapsed/refractory ALL conducted through the Therapeutic Advances in Childhood Leukemia and Lymphoma (TACL) Consortium was closed early for unacceptable toxicity.

A question considered by the care teams of both patients reported here was whether epigenetic therapy could have dual effect, treating both the relapsed solid tumor as well as the secondary MDS/AML. Preclinical work has suggested that both DNMT and HDAC inhibitors display anti-neoplastic activity in certain solid tumors, including neuroblastoma and rhabdomyosarcoma. For example, the pro-apoptotic gene, caspase 8, was found to be hypermethylated in neuroblastoma cell lines, leading to loss of gene expression. Exposure to decitabine restored caspase-8 expression, increasing sensitivity to cytotoxic chemotherapy [29, 30]. Similarly, the cyclin-dependent kinase inhibitor *p21 WAF1 (CDKN1A)* was found to be methylated and silenced in primary rhabdomyosarcoma tumors but could be reactivated by decitabine [31]. Furthermore, HDAC1 inhibition was shown to sensitize multidrug resistant neuroblastoma cell lines to cytotoxic drugs and to induce cell death in human rhabdomyosarcoma cell lines [32–34]. HDAC inhibition has also been shown to induce differentiation in neuroblastoma *in vitro* [35]. Based on these preclinical findings, a phase I study was conducted examining low-dose decitabine combined with doxorubicin and cyclophosphamide for children with relapsed neuroblastoma and other solid tumors. Results showed that this combination was safe and tolerable and a third of patients had stable disease for 4 cycles [16]. Another study examined single agent vorinostat with and without 13-cis retinoic acid for children with relapsed/refractory solid tumors, showing that the drug was safe and tolerable and led to prolonged stable disease in a small subset of patients [12]. It is difficult to assess whether the epigenetic therapy had any stabilizing effect on Patient 1's solid tumor disease since disease evaluation scans were not pursued in the setting of palliative care. Her symptomatology at end-of-life may have been due to progression of solid tumor disease, refractory AML or both. Patient 2 experienced rapidly progressive solid tumor disease after discontinuation of oral etoposide, suggesting no significant effect of the epigenetic therapy on its own against her solid tumor disease.

In summary, we report two pediatric patients who developed secondary MDS/AML concurrent with solid tumor relapse who benefited from epigenetic combination palliative therapy with decitabine and vorinostat, experiencing stabilization of marrow disease and reasonable quality of life. Secondary MDS/AML is rare in children and rarer still to occur concurrently with solid tumor relapse. Therapy options are limited for these patients. Further research is needed to better elucidate the mechanism of action of these drugs in targeting therapy-associated MDS/AML. Given our experience, a prospective clinical trial is warranted to evaluate clinical response, measure biomarkers, and objectively assess quality-of-life measures.

Acknowledgments

Drs. Chana Glasser, Shakeel Modak and Julia Glade Bender would like to acknowledge the support of the Band of Parents.

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