Published in final edited form as:

Leuk Lymphoma. 2016 October ; 57(10): 2441–2444. doi:10.3109/10428194.2016.1138295.

A phase II study of the efficacy, safety, and determinants of response to 5-azacitidine (Vidaza®) in patients with chronic myelomonocytic leukemia

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Chronic myelomonocytic leukemia (CMML) is a hematopoietic stem cell neoplasm with features of both myelodysplasia and myeloproliferation.[1] Cytopenias and progression to acute myeloid leukemia (AML) account for majority of the morbidity and mortality in CMML. CMML is clinically heterogeneous with poor prognosis and median survival is approximately 2.5 years from diagnosis.[2] The risk of progression to AML and overall survival (OS) is correlated with certain clinical and hematological parameters, cytogenetic abnormalities, and gene mutations such as *ASXL1*. [2–5] Additionally somatic mutations in several genes correlate with adverse outcomes, although no consistent picture has emerged thus far. Stem cell transplantation offers a potential cure and reduced intensity conditioning regimens make this option available to older patients, but relapse rates remain high.[6,7] Transplant ineligible patients are typically managed with supportive care and/or hydroxyurea to alleviate symptoms related to myeloproliferation and splenomegaly.[8] Hypomethylating agents (HMAs), 5-azacitidine, and decitabine received regulatory approval for myelodysplastic syndromes (MDSs), based on two separate phase 3 studies.[9,10] Both studies included a small number of CMML patients, which formed the basis for the clinical use of HMAs in CMML. Several studies predominantly retrospective reported overall response rates of 20–70% and median survival ranging from 12 to 37 months (Supplementary Table 1). Prospective trials of 5-azacitidine specifically in CMML patients are limited and predictors of response are unknown. We designed a prospective non-randomized phase 2 study to determine safety and efficacy of 5-azacitidine in CMML, and to identify predictive biomarkers. The study (NCT01350947) was conducted at University of Utah Huntsman Cancer Institute and Oregon Health and Science Knight Cancer Institute.

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Supplemental data for this article can be accessed at <http://dx.doi.org/10.3109/10428194.2016.1138295>.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at <http://dx.doi.org/10.3109/10428194.2016.1138295>.

Institutional Review Board of each institution approved the study. Informed consent was obtained prior to enrollment in accordance with the Declaration of Helsinki. Celgene Corporation supported the trial. Inclusion criteria were age ≥ 18 years with a diagnosis of CMML, ECOG performance status ≤ 2 and adequate organ function. Progression to AML ($\geq 20\%$ blasts in the blood or bone marrow) or prior therapy with HMAs was not allowed (See Supplementary Appendix). Primary objective was to determine the rates of hematologic response (complete response, CR; partial response, PR; stable disease, SD; and hematologic improvement, HI) according to the IWG 2006 criteria.[11] The secondary objectives were to determine OS and progression free survival (PFS) at 24 months follow-up. As an exploratory objective, targeted sequencing of 5-azacitidine naïve samples was performed by next generation sequencing (NGS) to establish correlations between somatic mutations and response to 5-azacitidine (Methods, supplementary Appendix). 5-Azacitidine at a dose of 75 mg/m² was given by subcutaneous (sc) or intravenous (iv) routes for days 1–7 every 28-days, for at least 8 cycles unless progression to acute leukemia ($\geq 20\%$ blasts) or continuation of treatment was not determined to be in patient's best interest. All patients received 100% dose for cycle 1, dosing for subsequent cycles was adjusted utilizing an algorithm based on nadir counts, hematologic response, bone marrow cellularity, and renal toxicity (Supplementary Figure 1). Patients with an objective hematologic response at completion of cycle 8 (CR, PR or HI) or deemed to be benefiting from therapy by treating physician were continued for a maximum of 24 cycles. All other patients including those with stable disease but no objective response were discontinued. Adverse events were graded as per the CTCAEv4.0. Response evaluations were performed by bone marrow biopsy prior to cycle 2, cycle 4, and cycle 7. For patients continuing on study beyond 12 months, bone marrow biopsies were obtained prior to cycles 13 and 17, at the end of study or at progression, whichever came first. All patients were followed for at least 24 months for survival outcomes. OS and PFS were analyzed by utilizing the Kaplan–Meier method. Between November 2010 and December 2012, 11 patients were enrolled, of whom six were men and five were women. Median age was 69 years (range 56–85 years). Demographics are reported in Table 1. Prior treatments were hydroxyurea in one patient and another patient received ruxolitinib due to an incorrect initial diagnosis of primary myelofibrosis. Median number of cycles administered was 6 (range 1–24). Five patients received the planned minimum of 8 cycles: two patients received 24 cycles, one patient discontinued after 18 cycles due to cytopenias, and two discontinued after 18 and 22 cycles due to progressive disease without transformation to AML. Six patients discontinued study medication before completing 8 cycles: two progressed to AML after 5 and 6 cycles, respectively, three proceeded SCT and one due to cytopenias. Overall best hematologic response rates were: CR in three (27%), SD in four (36%), marrow CR in two (18%), progression to AML in one (9%); response was not evaluable in one patient who discontinued therapy before completing 3 cycles. Overall, two patients progressed to AML; one demonstrated primary resistance without any response and the second progressed after an initial stable disease. At 24 months, five patients had died including two patients who succumbed to complications of SCT. At two years, OS was 55% and median OS and PFS were 30 and 14.1 months, respectively [Figure 1]. Treatment was given on outpatient basis. Dose reductions were performed as per predefined protocol (Supplementary Figure 1). Most common treatment related side effects were cytopenias: grade 3 or 4 neutropenia in four patients (36%); grade 3 or 4 anemia in four patients (36%),

grade 3 or 4 thrombocytopenia in one patient (9%). Dose reduction or interruption due to cytopenias was required for at least 1 cycle in six patients (54%). Total number of 5-azacitidine cycles administered was 135; 61 (45%) of which required dose reduction. One patient (9%) experienced grade 3 GI bleeding and epistaxis. Targeted sequencing of a panel of 53 genes revealed at least two somatic mutations in each patient (Supplementary Tables 2 and 3). *TET2* and *SRSF2* were mutated in 63% ($n = 7$) and *ASXL1* was mutated in 45% ($n = 5$). *TET2* mutations occurred frequently in association with splicing gene mutations (*SRSF2* in 5 and *ZRSR2* in 1). Response rates to 5-azacitidine in CMML patients were not reported separately in the randomized studies.[9,10] One of seven patients (17%) treated with decitabine had a response compared to no responses in the best supportive care.[10] Subsequently, studies reported variable response and outcomes in CMML patients treated with HMAs (Supplementary Table 1). In a prospective trial of decitabine (included up to 29% marrow blasts), ORR was 38% with four CRs (10%). OS was 48% at two years and median OS was 18.5 months.[12] The UK MDS group prospectively studied the efficacy of 5-azacitidine in CMML. ORR was 20% by IWG criteria and median OS was 16 months. Majority of patients were at high risk, which may explain poor ORR. Our results were comparable to previously published reports. Notably, a predefined stringent algorithm was followed to avoid arbitrary dose adjustments. Our study was limited by poor accrual with only 11 enrolled of planned 50 patients. Major obstacles were the rarity of CMML, inconvenience of receiving treatment available on label on study, and reluctance to initiate therapy in asymptomatic elderly patients. These difficulties underscore the importance of multicenter studies in rare malignancies like CMML. Finally, predictive factors of response to HMAs in CMML are unknown. Cytogenetic abnormalities are present in ~30% of patients and have a strong prognostic impact.[3] All of our patients had a normal karyotype. Majority of CMML patients have recurrent somatic mutations on NGS.[13] Mutations in diverse cellular pathways were described including signal transduction (*KRAS*, *NRAS*, *CBL*, *JAK2*), epigenetic regulation (*TET2*, *DNMT3A*, *ASXL1*, *EZH2*, *IDH1/2*), transcriptional regulation (*RUNX1*), and mRNA splicing (*SRSF2*, *UTX*). *TET2*, *ASXL1*, and *SRSF2* mutations are most frequent (~50%), followed by *RUNX1* (10%).[14] The prognostic relevance of some somatic mutations is unclear, but *ASXL1* mutations are consistently associated with poor outcomes.[4,5] Little is known about the molecular determinants of response to HMAs in CMML. In two separate cohorts, somatic mutations were not predictive of response to treatment.[12,15] Using gene expression analysis, Braun et al. found that lower *CJUN* and *CMYB* gene expression was independently predictive of improved OS.[12] In a more recent analysis of patients treated with decitabine, NGS identified differential methylated regions (DMRs) between responders and nonresponders. DMRs were primarily localized to nonpromoter and distal regulatory enhancer regions. Transcriptional analysis confirmed up-regulation of genes associated with cell cycle in responders, whereas chemokines, *CXCL4*, *CXCL7*, and integrin $\beta 3$ were exclusively overexpressed in resistant patients.[15] In another analysis of a large Mayo-French cohort, age <65 years, *ASXL1* and *SRSF2* mutations negatively impacted survival. Predictors of response to HMAs were not reported.[16] Similar pattern of somatic gene mutations and frequencies were observed in our cohort. All three patients who obtained CR were *TET2* mutated, and responses observed in four other *TET2* mutated patients (three SD and one marrow CR). Interestingly, two patients who progressed to AML were positive for *SRSF2*

mutation. The significance of these observations is unclear given the small sample size emphasizing the need to study CMML in larger multicenter trials. Our results support the use of HMAs as first line in CMML patients requiring treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank all the patients and their families for participating in the study, nurses, and clinical research coordinators.

Funding information

This work was supported by a Specialized Center of Research Program Award (GCNCR0314A-UTAH to M.W.D) from The Leukemia & Lymphoma Society (LLS) and by The V Foundation for Cancer Research (to M.W.D). S.K.T is a recipient of 2013 Research Training Award for Fellows from the American Society of Hematology (ASH). J.S.K is a Special Fellow of the LLS and was supported by a Translational Research Training in Hematology Award from the ASH.

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Figure 1.
Kaplan–Meier survival estimates: progression free survival (PFS) and overall survival (OS).

Table 1

Patient characteristics, 5-azacitidine administration, and best response.

ID	Age (years)	Gender	ECOG PS	Prior therapy	WBC count (k/ μ l)	Hb (g/dL)	Platelet (k/ μ l)	PB Mon%	PB blasts%	BM blast%	LDH (U/L)	WHO subtype	CPSS	Mutation(s)	Route of 5-aza	Cycles of 5-aza	Best HR	Last follow up status
1-001	74	Female	0	None	20	14.1	118	33%	<1%	1%	512	CMML-1	Int-1	KRAS, SRSF2, TET2	sc	24	CR	Alive
1-002	85	Female	1	None	8.6	10.6	31	20%	0%	4.30%	457	CMML-1	Low	PHF6, RUNX1, TET2	sc	18	SD	Alive
1-003	56	Male	0	None	44	13.4	197	3%	0%	0%	436	CMML-1	Int-2	CSF3R, RAD21	iv	5	SD	Dead
1-004	69	Female	0	Ruxolitinib	19	13.6	285	6%	0%	1.20%	487	CMML-1	Int-2	ASXL1, CBL, SRSF2, TET2	iv	24	CR	Alive
1-005	65	Male	2	None	248	7.8	58	14%	0%	8.40%	1176	CMML-1	High	ASXL1, BRINP3, GATA2, PTPN11, RUNX1, SRSF2	iv	1	N/E	Alive
2-001	70	Female	2	None	58	12.7	108	28%	0%	14%	267	CMML-2	Int-2	ASXL1, CBL, SRSF2, TET2	iv	22	SD	Dead
2-002	57	Male	0	None	29	13.6	97	29%	0%	18%	191	CMML-2	Int-2	KRAS, SRSF2, TET2	iv	4	Marrow CR	Dead
2-003	78	Male	1	Hydroxyurea	3.8	8.1	29	47%	1%	7.50%	105	CMML-1	Int-2	CBL, SRSF2, TET2	iv	5	SD	Dead
2-004	70	Male	0	None	62	12.1	132	22%	0%	<20%	335	CMML-2	Int-2	RUNX1, SRSF2	iv	6	PD	Dead
2-005	61	Female	1	None	20	12.2	353	16%	0%	4%	292	CMML-1	Int-1	ASXL1, SETBP1	iv	6	Marrow CR	Alive
2-006	67	Male	2	None	34	10.7	126	36%	0%	0%	226	CMML-1	Int-1	ASXL1, NRAS, RUNX1, TET2, ZRSR2	iv	20	CR	Alive

ECOG PS, eastern cooperative oncology group performance status; WBC, white blood cell; Hb, hemoglobin; PB, peripheral blood; BM, bone marrow; LDH, lactate dehydrogenase; WHO, world health organization; CPSS, CMML specific risk score; BM, bone marrow; 5-aza, 5-azacytidine; HR, Hematologic response; CR, complete remission; mCR, marrow complete remission; N/E, not evaluable; SD, Stable disease; PD, progressive disease.