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Letters to *Blood*

TO THE EDITOR:

Treatment with 5-azacytidine induces a sustained response in patients with angioimmunoblastic T-cell lymphoma

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Peripheral T-cell lymphomas (PTCLs) are heterogeneous diseases resulting from the malignant transformation of mature T or natural killer cells. Their epidemiology varies widely, but PTCLs that derive from T follicular helper (TFH) cells, which include angioimmunoblastic T-cell lymphoma (AITL), follicular PTCL, and other nodal PTCLs with a TFH phenotype,[1] appear to be frequent among PTCLs.[2,3] Patients have a poor prognosis, especially after relapse, with a median overall survival (OS) of ~6 months.[4] The US Food and Drug Administration has approved pralatrexate, romidepsin, and belinostat for relapsing/refractory PTCL, but these 3 drugs still show limited activity in PTCL, with an overall response rate of ~30%.[5-7] Thus, PTCL therapy is still an unmet medical need.

Recurrent mutations in *TET2*,[8-10] *DNMT3A*,[9-11] and/or *IDH2*[9,10,12] are detected in ~80%, 25%, and 25% of patients with TFH-derived PTCL, respectively. These 3 genes directly or indirectly regulate cytosine methylation and hydroxymethylation, and mutations of these genes result in changes in DNA methylation levels.[13] *TET2*, *DNMT3A*, and *IDH1/2* are also mutated in myeloid neoplasms, especially acute myeloid leukemia and myelodysplastic syndromes.[13] Treatment with the hypomethylating agents (HMAs) 5-azacytidine and decitabine shows efficacy in these diseases, and the response rate to HMAs appears to correlate with *TET2*, *IDH1/2*, and/or *DNMT3A* mutations.[14-16] This suggests that HMAs could have activity against TFH-derived PTCL. We previously reported 2 patients with AITL and chronic myelomonocytic leukemia (CMML) who experienced sustained complete remission of both diseases after treatment with 5-azacytidine.[17,18] Here, we expand these observations and describe the response and outcome of a retrospective series of 12 AITL patients who received 5-azacytidinefor concomitant myeloid neoplasm or used as compassionate therapy in relapsing/refractory AITL patients in the absence of available therapy or when such therapy was contraindicated, at the discretion of the physician.

Patients received 5-azacytidine 75 mg/m^2 daily, subcutaneously, for 7 consecutive days, every 28 days, until progression or unacceptable toxicity. Evaluations were performed by computed tomography scan, and responses were assessed by investigators following the 1999 Cheson criteria.[19]

Two patients (PTCL1[17] and PTCL7[18]) were previously reported. AITL diagnoses were all confirmed by expert pathologists in the framework of the national program "lymphopath,"[3] based on the criteria of the World Health Organization 2008 classification, and tumor samples were collected in the frame of the Tenomic network from the Lymphoma Study Association. *TET2*, *IDH2*, *DNMT3A*, and *RHOA* status was centrally determined by targeted deep sequencing of DNA extracted from formalin-fixed, paraffin-embedded samples using PGM technology (ThermoFisher) (see supplemental Methods, available at the *Blood* Web site). The median depth of sequencing was 1432×.

Patient characteristics are given in Table 1. The median age was 70.5 years (interquartile range [IQR], 67.5-73.5 years). All but one had relapsed-refractory disease, with a median of 2 lines of therapy (IQR, 1.75-3), including cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)–like therapy in 10 cases and mini-CHOP in one. PTCL1 was the only treatment-naive patient. She received 5-azacytidine as first-line therapy for concomitant CMML with excess blasts and thrombocytopenia. Three other patients had asymptomatic CMML, and 1 patient had refractory cytopenia with multilineage dysplasia. In total, 5 out of 12 patients (41%) had an associated myeloid neoplasm. All patients had disseminated (stage III-IV) disease, and 4 out of 12 patients (33%) had a poor performance status (Eastern Cooperative Oncology Group [ECOG] >2) at 5-azacytidine initiation.

Treatment was introduced between January 2013 and July 2016. Patients received a median of 5.5 cycles (IQR, 3.75-17 cycles). In addition to 5-azacytidine, 6 out of 12 patients (50%) received rituximab because of the presence of Epstein-Barr virus replication or numerous Epstein-Barr virus B-blasts in the lymph node biopsy.

Treatment was well tolerated. Three patients required transfusion, and none developed febrile neutropenia. One patient experienced grade 2 neuropathy that was considered to be

Table 1. Patient characteristics and treatment

ID	Sex	Age at diagnosis (y)	Associated myeloid disorder	Mutations (VAF%)	IPI at diagnosis	Number of previous therapies	Previous auto-HSCT	Stage	LDH	ECOG	Rituximab (no. of cycles)	5-azacytidine (no. of cycles)	Best response
PTCL1	F	79	CMML	TET2 p.Q891fs (34.6) TET2 c.3955-2A>G (38.4)	4	0	0	3	1	2	4	61	CR
PTCL2	M	69	MDS	TET2 p.I249fs (30)	3	1	0	4	0	2	4	25	CR
PTCL3	F	70	No	TET2 p.C1271W (82) RHOA p.K18N (11.4)	2	6	0	3	0	1	0	7	CR
PTCL4	M	75	CMML	TET2 p.E1879A (23)	2	4	0	3	1	3	0	6	CR
PTCL5	M	63	No	TET2 p.H1904R (42.2) TET2 p.E1207K (44) DNMT3A p.G707fs (40.20%)	4	2	1	4	1	1	6	5	CR
PTCL6	F	73	No	TET2 p.R1465X (28.3) DNMT3A p.R882H (30.6) RHOA p.G17V (24.3)	4	2	0	4	1	4	8	16	PR
PTCL7	F	85	CMML	TET2 p.S835X (27.6) TET2 c.3594+1G>C (24.7) DNMT3A p.R882H (27) RHOA p.G17V (2.8)	3	1	0	4	0	4	0	20	CR
PTCL8	M	39	No	TET2 p.T938fs (30.3) RHOA p.G17V (29.10)	4	2	0	4	1	1	6	4	SD
PTCL9	F	81	CMML	TET2 p.R1404X (30) TET2 p.C1298Y (31) RHOA p.G17V (12.10)	3	3	0	4	1	1	0	4	PR
PTCL10	M	51	No	TET2 p.R1216X (11.3) TET2 p.C1271W (8.9) IDH2 p.R172K (2.2)	2	3	1	3	1	1	0	3	PR

F, female; HSCT, hematopoietic stem cell transplant; IPI, International Prognostic Index; M, male, MDS, myelodysplastic syndrome; SD, stable disease; VAF, variant allele frequency.

Table 1. (continued)

ID	Sex	Age at diagnosis (y)	Associated myeloid disorder	Mutations (VAF%)	IPI at diagnosis	Number of previous therapies	Previous auto-HSCT	Stage	LDH	ECOG	Rituximab (no. of cycles)	5-azacytidine (no. of cycles)	Best response
PTCL11	M	71	No	TET2 p.H1904L (24.8) DNMT3A p.R882H (45.3)	5	2	0	4	1	2	6	2	SD
PTCL12	M	69	No	TET2 p.L1244fs (28.3) TET2 p.H937fs (10.2)	5	2	0	4	1	4	0	2	SD

F, female; HSCT, hematopoietic stem cell transplant; IPI, International Prognostic Index; M, male, MDS, myelodysplastic syndrome; SD, stable disease; VAF, variant allele frequency.

paraneoplastic syndrome unrelated to treatment, and another experienced unexpected digestive toxicity (grade 3 diarrhea). There were no treatment-related deaths.

Nine patients showed a response, including 6 complete responses (CRs) and 3 partial responses (PRs), leading to an overall response rate of 75%, including 50% CRs and 25% PRs (Figure 1A). After a median follow-up of 27 months, the median progression-free survival was 15 months and median OS of 21 months (Figure 1 B-C). No patient died of myeloid neoplasm during the follow-up period. It is noteworthy that some elderly patients with poor performance status (ECOG 3-4) had a sustained AITL response after 5-azacytidine treatment, with an acceptable tolerance.

Three patients stopped treatment after 5, 7, and 6 cycles: 1 because of digestive toxicity, 1 at his request, and 1 at the request of the physician. The first 2 patients remained in complete remission >18 months after discontinuing 5-azacytidine, whereas the last (PTCL4) relapsed 9 months after. Treatment was resumed, rapidly resulting in a sustained CR. He is currently still on therapy.

Five patients have shown a sustained response, as they are still in complete remission >23 months after treatment initiation. Three are still receiving treatment, whereas it was discontinued in the other 2 patients, raising the question of the optimal duration of HMA therapy and the possibility of discontinuing treatment.

Molecular studies were centrally performed using targeted deep sequencing. We detected *TET2* mutations in 12 out of 12 patients, and 7 out of 12 patients (58%) had 2 mutations. In addition, 4 out of 12 patients (33%) had *DNMT3A* mutations, 5 out of 12 patients (41%) had *RHOA* mutations, 4 out of 5 patients had a p.G17V substitution, and only 1 patient had an *IDH2*^{R172} mutation (supplemental Table 1). We were unable to assess the impact of *TET2* mutation on treatment response because the samples from all patients were *TET2* mutated. Within the limit of this cohort, we observed no relationship among number of *TET2* mutations; mutations in *DNM3A*, *IDH2*, or *RHOA*; and response to treatment. An association with rituximab was not associated with a higher response rate (66% vs 83%; $P = 1$), likely reflecting the limited efficacy of this drug in AITL.[20] Finally, the 5 patients with an associated myeloid neoplasm responded (4 CRs and 1 PR), while 4 out of 7 patients without associated myeloid anomalies responded (2 CRs and 2 PRs; $P = .2$), indicating that the effect of 5-azacytidine on AITL is not restricted to patients with associated myeloid disease.

We highlight here an association of AITL with CMML in 4 patients. This association has rarely been reported[17,18,21] but could be more frequent than previously thought. Indeed, these 2 neoplasms share common oncogenic events, such as *TET2* or *DNMT3A* mutations, which usually occur in hematopoietic progenitor cells and could lead to the development of both diseases. Clinical and molecular studies are required for a better understanding of this association. Furthermore, the mechanism of action of 5-azacytidine in AITL has not been elucidated yet. Indeed, it is unknown whether 5-azacytidine has a direct effect on neoplastic T cells or whether it acts on abnormal TET2-mutated myeloid cells. Indeed, it could be hypothesized that abnormal *TET2*-mutated myeloid cells could

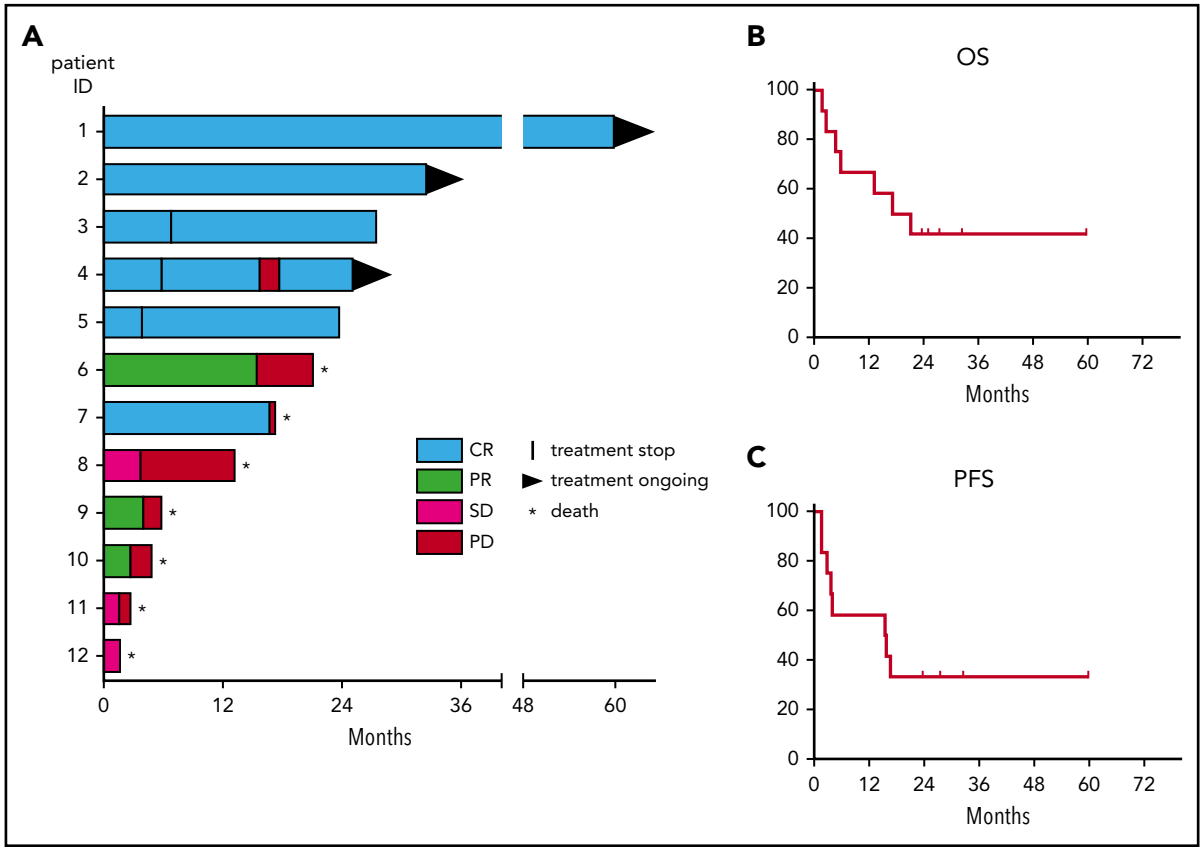

Figure 1. Patient outcomes after 5-azacytidine treatment. (A) Best response and duration of response are shown on the swimmer plot. Blue indicates a complete response, green a partial response, violet stable disease (SD), and pink progressive disease (PD). Stars indicate deceased patients, arrows ongoing treatment, and vertical lines the cessation of treatment. (B) Kaplan-Meier curve showing overall survival. (C) Kaplan-Meier curve showing progression-free survival (PFS).

provide signals promoting survival and expansion of neoplastic T cells, which would be reversed by 5-azacytidine.

Several other questions remain unanswered, especially whether TFH-derived PTCLs other than AITL, which share similar mutational anomalies with AITL (especially recurrent *TET2*, *DNMT3A*, and *RHOA* mutations),[22] respond similarly to 5-azacytidine or whether genetic or epigenetic response markers could be determined to predict which patients could benefit from this treatment. These questions warrant a prospective study, which is planned to start this year (EudraCT #2017-003909-17).

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Authorship

Contribution: F.L., O.H., P.G., and R.D. designed the study and wrote the manuscript; F.L., J.D., P.S., O.T., M.C., C.S., A.M., C.H., O.H., and R.D. treated the patients and approved the manuscript; V.F. collected cases and material and approved the manuscript; and F.L., P.S., C.R., L.P., and P.G. performed the histological and molecular studies and approved the study.

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Footnotes

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TO THE EDITOR:

Germline loss-of-function *SAMD9* and *SAMD9L* alterations in adult myelodysplastic syndromes

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Familial bone marrow failure (BMF) syndromes present typically in children and younger adults.[1-3] A number of germline (GL) mutations in genes such as *DDX41*,[4] *RUNX1*,[5] *ETV6*,[6] *GATA2*,[7] and *ANKRD26*[8] have been implicated in the pathogenesis of familial myelodysplastic syndromes (MDSs) and define a disease class of myeloid neoplasms with GL predisposition.[9] GL *SAMD9* mutations arise in MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) syndrome patients,[10,11] whereas GL *SAMD9L* mutations occur in pediatric MDS and BMF patients.[7,12,13] *SAMD9* and *SAMD9L* are proximal on 7q21.2. GL variants in these genes enhance their physiologic growth-inhibitory function and are thus gains of function (GOF).[12,14,15] Supporting this notion, somatically acquired −7/del(7q), or loss-of-function (LOF) missense and truncating mutations affecting the same *SAMD9/SAMD9L* mutant allele, revert their GL mutation to escape its inhibitory effects.[10-12,15-17] We report a 9-month-old infant with familial thrombocytopenia with a *SAMD9L* variant, marrow normocellularity, and the absence of megakaryocytes, which resolved following 4 months of transfusion support (supplemental Figure 1; available on the *Blood* Web site). Whole exome sequencing (WES) identified a novel heterozygous GL variant in a conserved amino acid region of *SAMD9L* (Trp517Arg) with likely deleterious consequences predicted by in silico analysis (supplemental Table 1). Sequencing of the patients' families confirmed that the GL variant was of paternal origin. Although *SAMD9* and *SAMD9L* GL