A non-cytotoxic regimen of decitabine to treat refractory T-cell large granular lymphocytic leukemia

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Abstract

T-cell large granular lymphocytic leukemia (T-LGL) is a chronic malignancy characterized by clonal expansion of cytotoxic T-cells possibly complicated by cytopenias of one or more myeloid lineages. By describing a multiply-refractory T-LGL case, this report highlights the importance of molecular-targeted and non-cytotoxic therapies in the context of frail elderly patients.

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Abstract

T-cell large granular lymphocytic leukemia (T-LGL) is a chronic malignancy characterized by clonal expansion of cytotoxic T-cells possibly complicated by cytopenias of one or more myeloid lineages. By describing a multiply-refractory T-LGL case, this report highlights the importance of molecular-targeted and non-cytotoxic therapies in the context of frail elderly patients.

Key Clinical Message

We report on a novel, successful, non-toxic therapy to treat multiply-resistant T-cell large granular lymphocytic leukemia in an elderly patient.

Background

T-cell large granular lymphocyte leukemia (T-LGL) is a chronic lymphoproliferative disorder characterized by clonal expansion of cytotoxic T-cells and cytopenia of one or more myeloid lineages[1]. Despite a typically indolent nature, most patients eventually require treatment because of severe cytopenias, caused by either direct (cellular-mediated) or indirect (cytokines) attack of myeloid precursors by the clonal T-cells.[1,2]Because T-LGL in its severe form is a rare condition, no head-to-head comparisons of different treatment options have been conducted. Guided by single-arm studies, three drugs – methotrexate (MTX), cyclosporine (CsA), or cyclophosphamide (Cy) - are routinely used as first-line therapies. Overall response rates (ORR) have been reported at 38%-60% for MTX, 45%-92% for CsA and 47%-72% for Cy[2,3], and a recent retrospective analysis reported comparable ORR of 61.5%-74.4% for all three agents.[4] Even less data exist as to the management of refractory cases. Several drugs inhibiting epigenetic repressing enzymes of the histone deacetylase (HDAC) class are approved for treating other peripheral T-cell malignancies, and a case-report described the successful use of the HDAC-inhibitor belinostat (1000 mg/m^2 intravenously, days 1-5 in 21-day cycles) to produce red-cell transfusion independence in a patient with T-LGL and anemia refractory to MTX, CsA and Cy.[5] HDAC-inhibitors, however, cause cytopenias in their own right, via cytotoxic or cytostatic actions possibly inherent to HDAC-inhibition, which can undermine the cytopenia-correction goal-of-care. We describe here an elderly patient with T-LGL with red cell transfusion-dependence refractory to several lines of therapy including an HDAC-inhibitor, who was successfully treated with the drug decitabine administered by a regimen designed to inhibit/deplete the epigenetic repressing enzyme DNA methyltransferase 1 (DNMT1) without cytotoxicity.[6-9]

Case report

A 79-year-old man with coronary artery disease, chronic kidney disease (CKD) stage III and non-melanocytic skin cancer presented to his primary care physician in June 2019 with shortness of breath and decreased exercise tolerance. Initial work-up revealed macrocytic anemia (hemoglobin [Hb] 9.4 g/L; MCV 105 fL), absolute neutrophil count of 1.6x10^9/L, and normal platelet count (180x10^9/L). Abnormal large granular lymphocytes (LGL) were noted on blood smear and represented 80% of total circulating lymphocytes (absolute lymphocyte count 1.5x10^9/L). A bone marrow (BM) evaluation showed moderate erythroid hyperplasia, normal granulopoiesis and megakaryopoiesis, no dysplastic changes or chromosomal abnormalities, and infiltration by abnormal cytotoxic T-cells confirmed by flow cytometry (**Fig.1A**). Molecular studies revealed a canonical *STAT3* $^{\text{Y640F}}$ mutation (variant allelic frequency 11.5%) and a clonal T-cell rearrangement, confirming the diagnosis of T-LGL.[1,2]The anemia was progressive, resulting in red cell transfusion-dependence and prompting initiation of therapy with MTX 15 mg weekly. During 6 weeks of MTX therapy, fatigue worsened and red cell transfusion needs increased, leading to MTX discontinuation and the initiation of 2$^{\text{nd}}$-line therapy with prednisone 60 mg daily for 3 weeks with subsequent tapering, for a total duration of 8 weeks. Again, no response was observed, prompting initiation of 3$^{\text{rd}}$-line therapy with CsA 2 mg/kg per day in divided doses along with short-course of prednisone (40 mg daily orally tapered over 14 days). Over 4 months of CsA therapy, there was no decrease in red cell transfusion needs, and kidney toxicity required CsA dose-reduction. At this point, BM evaluation was repeated: morphological observations were similar to initial presentation - expanded erythropoiesis and T-LGL cell infiltration. Two cytogenetic abnormalities, not observed at initial presentation, were detected (-Y in 3/20, and +8 in 4/20 metaphases). Clonal hematopoiesis with -Y is

not uncommon in older individuals, and trisomy 8 has been reported in association with T-LGL[10,11], but since there were no dysplastic changes the diagnosis remained unchanged. CsA therapy was discontinued because of nephrotoxicity and lack of response and 4th-line therapy with tacrolimus (4 mg per day in divided doses) was initiated. As patient's erythropoietin level was inappropriately low in the context of anemia (90 mU/mL), epoetin alpha (60,000 units biweekly) was incorporated into therapy (as per indication of anemia caused by CKD). Red cell transfusion needs did not decrease after 12 weeks of this treatment, prompting the addition of MTX 10 mg weekly. After 12 weeks of tacrolimus, MTX and epoetin combination, the patient was still transfusion-dependent, therefore, the tacrolimus and MTX were discontinued and the HDAC-inhibitor vorinostat (400 mg *per os*daily), approved for the treatment of other peripheral T-cell malignancies, was initiated as 5th-line therapy. Over a 3 month period, this treatment was complicated by anorexia, diarrhea and worsening of fatigue, and did not register any improvement. The HDAC-inhibitor was thus discontinued and the patient was initiated on 6th-line therapy with a non-cytotoxic, DNMT1-targeting regimen of subcutaneous decitabine 0.2 mg/kg once a week. Red cell transfusion-independence was achieved around 7 weeks after initiation of this therapy, accompanied by subjective improvements in the patient's symptoms and sense of well-being with no side-effects (**Fig.1B**).

Discussion

Although a potential role for MTX to treat *STAT3* -mutated cases, which in general appear to have worse survival outcomes than wild-type counterparts, has been proposed[12,13], there is no consensus as to the optimal treatment algorithm for T-LGL, whether *STAT3* -mutated or otherwise.[1] A non-cytotoxic molecular-targeted therapy would be ideal, since the therapy aims at improving low blood counts, a goal that can be undermined by cytotoxicity, especially in elderly patients who have functional hematopoiesis reserves already diminished by age and disease processes. As a molecular target, DNMT1 has been (i) clinically validated for treating other T-cell malignancies; (ii) pre-clinically validated for the treatment of T-LGL[14]; and (iii) we had previously developed and described a dose, route-of-administration and schedule of decitabine to deplete DNMT1 without cytotoxicity to BM.[6-9] The non-cytotoxic, DNMT1-targeting mechanism-of-action of this regimen has been documented previously in patients with myeloid malignancies and β-hemoglobinopathies.[6-9]Taken altogether, these data prompted our first use of this decitabine regimen to treat an elderly patient with T-LGL refractory to multiple lines of therapy and red cell transfusion-dependence. The subjective and objective clinical response and safety profile, and the clinical responses to DNMT1-targeting reported also for other T-cell malignancies, suggest that non-cytotoxic DNMT1-targeting should be further evaluated to treat T-LGL.

DECLARATIONS

All procedures were carried out in accordance with guidelines set forth by the Declaration of Helsinki.

Competing interests: Ownership: YS – EpiDestiny. Income: none. Research support: none. Intellectual property: YS - patents around tetrahydrouridine, decitabine and 5-azacytidine (US 9,259,469 B2; US 9,265,785 B2; US 9,895,391 B2) and cancer differentiation therapy (US 9,926,316 B2).

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References

1. Lamy T, Moignet A, Loughran TP, Jr. LGL leukemia: from pathogenesis to treatment.*Blood.* 2017;129(9):1082-1094.

2. Sanikommu SR, Clemente MJ, Chomczynski P, et al. Clinical features and treatment outcomes in large granular lymphocytic leukemia (LGLL). *Leuk Lymphoma.* Feb 2018;59(2):416-422.

3. Moignet A, Lamy T. Latest Advances in the Diagnosis and Treatment of Large Granular Lymphocytic Leukemia. *Am Soc Clin Oncol Educ Book.* May 23 2018;38:616-625.

4. Dong N, Castillo Tokumori F, Isenalumhe L, et al. Large Granular Lymphocytic Leukemia - a Retrospective Study of 319 Cases. *Am J Hematol.* Apr 5 2021.

5. Poh C, Arora M, Ghuman S, Tuscano J. Belinostat in Relapsed/Refractory T-Cell Large Granular Lymphocyte Leukemia. *Acta Haematol.* 2021;144(1):95-99.

6. Awada H, Mahfouz RZ, Kishtagari A, et al. Extended experience with a non-cytotoxic DNMT1-targeting regimen of decitabine to treat myeloid malignancies.*British Journal of Haematology.* 2020;188(6):924-929.

7. Sauntharajah Y. Key clinical observations after 5-azacytidine and decitabine treatment of myelodysplastic syndromes suggest practical solutions for better outcomes. *Hematology Am Soc Hematol Educ Program.*2013;2013:511-521.

8. Sauntharajah Y, Hillery CA, Lavelle D, et al. Effects of 5-aza-2'-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. *Blood.* Dec 1 2003;102(12):3865-3870.

9. Sauntharajah Y, Sekeres M, Advani A, et al. Evaluation of noncytotoxic DNMT1-depleting therapy in patients with myelodysplastic syndromes. *J Clin Invest.* Mar 2 2015;125(3):1043-1055.

10. Loughran TP, Jr., Kadin ME, Starkebaum G, et al. Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. *Ann Intern Med.* Feb 1985;102(2):169-175.

11. Durrani J, Awada H, Kishtagari A, et al. Large granular lymphocytic leukemia coexists with myeloid clones and myelodysplastic syndrome. *Leukemia.* 2020/03/01 2020;34(3):957-962.

12. Shi M, He R, Feldman AL, et al. STAT3 mutation and its clinical and histopathologic correlation in T-cell large granular lymphocytic leukemia. *Human Pathology.*2018/03/01/ 2018;73:74-81.

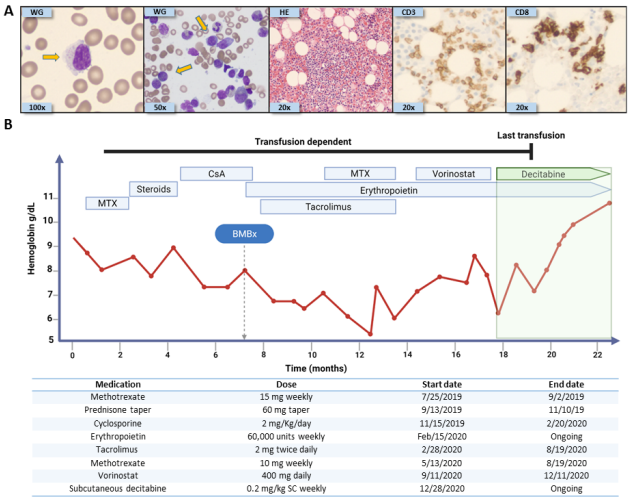
13. Barilà G, Teramo A, Calabretto G, et al. Stat3 mutations impact on overall survival in large granular lymphocyte leukemia: a single-center experience of 205 patients. *Leukemia.* 2020/04/01 2020;34(4):1116-1124.

14. Teramo A, Gattazzo C, Passeri F, et al. Intrinsic and extrinsic mechanisms contribute to maintain the JAK/STAT pathway aberrantly activated in T-type large granular lymphocyte leukemia. *Blood.* May 9 2013;121(19):3843-3854, S3841.

Figure legend.

Figure 1.Histopathological findings and treatment course of our index case. A) From left to right: Wright-Giemsa (WG) stain of peripheral blood showing a classical large granular lymphocyte (100X); WG stain of bone marrow (BM) aspiration showing classical LGL (50X); hematoxilin-eosin stain (20X) of the BM biopsy core with infiltration of small lymphocytes characterized by CD3 (20X) and CD8 (20X) positivity by immunostaining. Flow cytometry confirmed the aberrant T-LGL phenotype: CD2, CD3, CD5 dim, CD8, CD16, and CD57. Yellow arrows indicate large granular lymphocytes. **B)** Timeline depicting the type of treatment received along with duration of administration. On the bottom details on treatments doses.

MTX: Methotrexate, CsA: Cyclosporine, BMBx: Bone marrow biopsy



Medication	Dose	Start date	End date
Methotrexate	15 mg weekly	7/25/2019	9/2/2019
Prednisone taper	60 mg taper	9/13/2019	11/10/19
Cyclosporine	2 mg/Kg/day	11/15/2019	2/20/2020
Erythropoietin	60,000 units weekly	Feb/15/2020	Ongoing
Tacrolimus	2 mg twice daily	2/28/2020	8/19/2020
Methotrexate	10 mg weekly	5/13/2020	8/19/2020
Vorinostat	400 mg daily	9/11/2020	12/11/2020
Subcutaneous decitabine	0.2 mg/kg SC weekly	12/28/2020	Ongoing