To the editor:

Hydroxyurea: a new old therapy for Langerhans cell histiocytosis


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Langerhans cell histiocytosis (LCH) is caused by lesions arising from myeloid dendritic cells that present in a variety of organ systems ranging from single bone or skin lesions to diffuse multisystem disease.1 Survival for patients with LCH limited to skin or bone is nearly universal, but many patients relapse and can require multiple therapies to achieve a durable remission. One retrospective review found that 40% of patients relapsed at least once.2 Patients who relapsed had a twofold increased risk of permanent consequences such as diabetes insipidus and neurodegenerative disease. Skin-only LCH has a favorable prognosis and can resolve spontaneously or respond to minimal chemotherapy. However, 24% of patients with multisystem disease who relapsed developed skin or other nonbone single-system disease, some relapsing with skin involvement a second time.2 Another institutional series reported that among patients with LCH limited to the skin who required treatment, 11% relapsed. Patients with skin and another disease site treated with vinblastine and prednisone have a 56% chance of relapse.3 Despite generally favorable outcomes in patients with skin LCH, a subset of patients develop chronic rashes or oral lesions that respond slowly to treatment with oral methotrexate (MTX), mercaptopurine, or prednisone and IV vinblastine or cytarabine. There is no clear standard of care for the management of cutaneous LCH. Similar to skin-only LCH, bone-only LCH is associated with minimal mortality, but patients with multiple bone lesions have increased rates of relapse and permanent consequences. Minkov et al found that single-system skeletal relapse was the most common site of recurrence.2 Twenty percent to 30% of patients with bony disease develop permanent orthopedic sequelae, such as vertebral collapse or facial asymmetry.4-6 In addition to complications specific to skin and bone LCH, patients with uncontrolled LCH or multiple relapses may develop long-term morbidities including chronic pain, endocrinopathies, and neurodegenerative symptoms.5

Given that the pathologic CD207+ dendritic cells in LCH arise from myeloid precursor cells driven by activating mutations in the MAPK pathway, we hypothesized that therapies directed against immature myeloid cells may be effective in the treatment of LCH. Hydroxyurea (HU) is a myelotoxic ribonucleotide reductase inhibitor with proven efficacy in other neoplasms of myeloid origin, such as chronic myelogenous leukemia, essential thrombocytopenia, and polycythemia vera.7,8 We report a single-center experience with HU or HU/MTX therapy in a cohort of LCH patients with skin and/or oral lesions, skin and bone, bone only, or lymph node involvement.

The charts of 15 patients treated at Texas Children’s Hospital from November 2013 to April 2016 were reviewed in accordance with institutional review board–approved protocols at Baylor College of Medicine. Patients were selected for HU therapy based on specific clinical characteristics: recurrent or refractory skin or bone disease, either alone or in combination with lymph node involvement, following standard-of-care frontline therapy or intolerance of other therapies. Treatment response was reported using modified Response Evaluation Criteria in Solid Tumors (RECIST), as applied to lymphomas, criteria: complete resolution (CR), partial resolution (PR), stable disease (SD), or progressive disease (PD).9 Bone lesion responses for those >10 mm on computed tomography, magnetic resonance imaging, or positron emission tomography (PET) scan were tracked with serial imaging. Unmeasurable lesions such as rashes were followed by changes in examination as documented by the physician. Pediatric patients were started at a dose of 20 mg/kg divided twice daily and adult patients were started at a dose of 500 mg twice daily. Five patients were treated with a combination of HU/MTX after PR or SD with HU alone. Methotrexate was given at 5 to 10 mg twice weekly. Treatment doses were titrated to maximum effect, with a target absolute neutrophil count of 1000 to 1500.

Fifteen patients with relapsed/refractory LCH of skin and bone (4), skin and/or oral only (8), bone only (2), and skin, oral, and lymph node (1) were identified for HU treatment, following previous treatment with 1 to 5 regimens. The median age at initiation of HU was 41.2 years, ranging from 2.5 to 73.2 years. The median duration of therapy was 10 months, ranging from 1 to 24 months (Table 1). In patients treated with HU alone, best responses achieved included CR in 6 of 14 patients (43%), PR in 5 of 14 patients (36%), SD in 2 (14%), and PD in 1 (7%). In 4 patients achieving PR on HU therapy alone, and in 1 patient who had previously achieved PR on MTX alone, HU was used in combination with MTX, resulting in eventual CR in 2 of these patients (Figure 1), for total response rates of 80% (55% CR, 27% PR). Following treatment with HU, 6 patients (40%) had progression of symptoms or relapsed after initial response. The median time to disease progression/relapse was 5.7 months, ranging from 1 to 17 months. Of the 6 patients, 4 (67%) progressed or developed recurrent symptoms while taking HU and 2 (33%) after stopping therapy; these 2 patients again achieved disease response with reinitiation of HU therapy. Grade 3-4 toxicity was limited to anemia and neutropenia in 1 patient requiring blood transfusion and dose reduction, and thrombocytopenia and neutropenia in a second patient, requiring temporary dose reduction, but with no serious infections or adverse events throughout the course of HU therapy.

This case series shows that HU has activity against LCH in many patients, as 12 of 15 patients (80%) had either partial or complete responses. Recent studies showing the pathologic cell of origin to be a myeloid dendritic cell rather than a terminally differentiated Langerhans cell have led to the recommendation that LCH be reclassified as a myeloid dendritic cell neoplasm.10-13 It is therefore not surprising that HU would be effective in the treatment of LCH given its proven efficacy in treating other myeloid disorders.8,14 Historically, adult patients have tolerated HU well. Use in children is also reasonable given extensive experience in the treatment of children with sickle cell disease has proven HU to be both well tolerated and safe for prolonged use.15,16 The toxicity profile of daily oral HU is favorable when compared with alternative salvage therapies such as cytarabine, cladribine, or clofarabine.17 Since the discovery of BRAF-V600E and other recurrent mutations in the MAPK signaling pathway,11,18-20...
<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Age at start of HU</th>
<th>Historical sites of disease</th>
<th>HU target lesion</th>
<th>Risk status</th>
<th>Prior therapies</th>
<th>Duration of HU therapy, mo</th>
<th>Grade 3-4 toxicities</th>
<th>Best response</th>
<th>Progressed/relapse</th>
<th>Concurrent chemotherapy</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>45 y 5 mo</td>
<td>48 y 7 mo</td>
<td>Bone Oral Skin</td>
<td>Skin Oral</td>
<td>LR</td>
<td>1. Radiation therapy 2. Cytarabine</td>
<td>3</td>
<td>No</td>
<td>PD</td>
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<td>N/A</td>
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<tr>
<td>2</td>
<td>F</td>
<td>33 y 10 mo</td>
<td>34 y 2 mo</td>
<td>Pulmonary Skin Oral Skin</td>
<td>Skin Oral</td>
<td>LR</td>
<td>1. Prednisone, azathioprine 2. MTX, 6-MP</td>
<td>1</td>
<td>No</td>
<td>SD</td>
<td>No</td>
<td>N/A</td>
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<tr>
<td>3</td>
<td>F</td>
<td>48 y 7 mo</td>
<td>58 y 2 mo</td>
<td>Pulmonary Skin Bone Pituitary</td>
<td>Skin Mastoid</td>
<td>LR</td>
<td>1. Cytarabine 2. MTX 3. Etoposide 4. Cytarabine 5. Campath</td>
<td>10</td>
<td>No</td>
<td>SD</td>
<td>No</td>
<td>N/A</td>
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<tr>
<td>4</td>
<td>M</td>
<td>12 y</td>
<td>28 y 5 mo</td>
<td>Skin Oral Pituitary</td>
<td>Skin Oral</td>
<td>LR</td>
<td>1. MTX 2. Narrow band UV 3. IV MTX</td>
<td>6</td>
<td>No</td>
<td>PR</td>
<td>No</td>
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<td>M</td>
<td>27 y 8 mo</td>
<td>33 y 2 mo</td>
<td>Skin</td>
<td>Skin</td>
<td>LR</td>
<td>1. MTX 2. Cytarabine, MTX 3. Etoposide</td>
<td>6</td>
<td>No</td>
<td>PR</td>
<td>Yes</td>
<td>MTX</td>
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<td>1 mo</td>
<td>26 y 8 mo</td>
<td>Spleen Liver Bone marrow</td>
<td>Oral</td>
<td>HR</td>
<td>1. Vinblastine, prednisone 2. Repeat vinblastine, prednisone</td>
<td>12</td>
<td>No</td>
<td>PR</td>
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<td>50 y 6 mo</td>
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<td>Bone Skin Oral Vaginal</td>
<td>LR</td>
<td>1. Prednisone, vinblastine 2. Prednisone, vinblastine, 6-MP, MTX 3. Cladribine 4. Cytarabine 5. MTX</td>
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<td>No</td>
<td>PR</td>
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<td>MTX</td>
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<td>47 y</td>
<td>73 y 2 mo</td>
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<td>1. Prednisone 2. Cytoxan 3. Etoposide</td>
<td>4</td>
<td>No</td>
<td>CR</td>
<td>No</td>
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<td>M</td>
<td>8 y</td>
<td>9 y 2 mo</td>
<td>Skin</td>
<td>Skin</td>
<td>LR</td>
<td>1. MTX + prednisone 2. 6-MP + MTX</td>
<td>6</td>
<td>Thrombocytopenia Neutropenia</td>
<td>CR</td>
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<td>1 y 4 mo</td>
<td>2 y 6 mo</td>
<td>Skull Sternum Spleen (by PET)</td>
<td>Bone</td>
<td>HR</td>
<td>1. Cytarabine 2. Local injection</td>
<td>8</td>
<td>No</td>
<td>CR</td>
<td>No</td>
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<tr>
<td>11</td>
<td>M</td>
<td>66 y 2 mo</td>
<td>69 y 8 mo</td>
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<td>Skin</td>
<td>LR</td>
<td>1. MTX 2. Prednisone</td>
<td>10</td>
<td>No</td>
<td>CR</td>
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<tr>
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<td>15 y 11 mo</td>
<td>19 y 1 mo</td>
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<td>Bone</td>
<td>LR</td>
<td>1. Cytarabine 2. Clofarabine 3. 6-MP/MTX</td>
<td>12</td>
<td>No</td>
<td>CR</td>
<td>No</td>
<td>N/A</td>
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<tr>
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<td>44 y</td>
<td>57 y 10 mo</td>
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<td>Skin Oral Vaginal Otic</td>
<td>LR</td>
<td>1. MTX/6-MP 2. MTX</td>
<td>17</td>
<td>No</td>
<td>CR</td>
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<td>33 y 1 mo</td>
<td>41 y 3 mo</td>
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<td>Skin Oral Vaginal</td>
<td>LR</td>
<td>1. Cytarabine 2. Cladribine</td>
<td>22</td>
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<td>CR</td>
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<td>58 y 6 mo</td>
<td>59 y 6 mo</td>
<td>Orbital Skin Oral</td>
<td>Skin Oral</td>
<td>LR</td>
<td>1. MTX/6-MP</td>
<td>24</td>
<td>Anemia Neutropenia</td>
<td>CR</td>
<td>No</td>
<td>MTX</td>
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</table>

6-MP, 6-mercaptopurine; CNS, central nervous system; F, female; HR, high risk; LR, low risk; M, male; mo, months; N/A, not applicable; ND, neurodegenerative LCH; Pit, pituitary; Pt, patient; y, years.
MAPK pathway inhibitors, such as vemurafenib, are another potential treatment option. However, these agents carry significant risk, including de novo squamous cell carcinoma in 20% to 25% of adults with melanoma, and little is known about the toxicity profile of long-term MAPK inhibition in pediatric patients. HU may prove to be a safe and effective therapeutic option for some LCH patients with the added benefits of low cost and ease of administration. In conclusion, our series showed HU alone or in combination with MTX to have promising activity in patients with multiply relapsed or refractory LCH, with 80% of patients having partial or complete responses with minimal toxicity. Strategies for future investigation include prospective trials randomizing HU against MTX for patients with skin-limited LCH; randomizing HU versus mercaptopurine as part of initial combination therapy for patients with high-risk organ involvement; or randomizing HU against observation alone as maintenance therapy for both low- and high-risk LCH.

*D.J.Z. and A.B.G. contributed equally.

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**References**


To the editor:

High incidence of activating STAT5B mutations in CD4-positive T-cell large granular lymphocyte leukemia

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Large granular lymphocyte (LGL) leukemia is a group of chronic lymphoproliferative disorders of cytotoxic T or natural killer (NK) cells frequently complicated with cytopenia and autoimmune phenomena.1,2 In the current World Health Organization (WHO) classification, T-LGL leukemia and chronic lymphoproliferative disorder of NK cells (CLPD-NK) are included in this category.3 Recurrent somatic mutations in the Src homology 2 (SH2) domain of the signal transducer and activator of transcription 3 (STAT3) gene have been found in T-LGL leukemia and CLPD-NK,4,5 leading to constitutive activation of STAT3 and dysregulation of genes downstream of STAT3. More recently, mutations outside the SH2 domain have been discovered in T-LGL leukemia.6 Activating mutations in the SH2 domain of the STAT5B gene were also identified in 2% of LGL leukemia patients,7 which further underlines the importance of the JAK/STAT signaling pathway in LGL leukemia.

The majority of T-LGL leukemia cases present with a clonal expansion of the CD8+ LGLs. However, in a small percentage of cases, the tumor cells have a CD4+ phenotype.5,10 Cytomegalovirus-derived stimulation and restricted use of the T-cell receptor (TCR)-Vβ region has been associated with CD4+ T-LGL cases,11 but this rare disease entity still remains poorly described. To further elucidate the pathogenesis of this rare subgroup of T-LGL leukemia, we explored the mutational landscape of CD4+ cases using exome and targeted amplicon sequencing. Patients diagnosed with T-LGL leukemia and CLPD-NK were recruited. The diagnostic criteria were based on the WHO classifications of 2008. Three patient cohorts (described in detail in the supplemental Appendix, available on the Blood Web site) were included in this study.

Exome sequencing was performed on 3 CD4+ T-LGL leukemia patients’ sorted tumor (CD4+ or CD4−CD8+ T cells) and control (CD4−) fractions. The exome was captured with Nimblegen SeqCap EZ Exome Library v2.0, and sequencing was performed with the Illumina HiSeq2000 sequencing platform. Candidate somatic mutations were identified with a bioinformatics pipeline described earlier,5 as well as a novel pipeline described in more detail in the supplemental Appendix. Through exome sequencing, we were able to identify novel somatic missense mutations in the transactivation domain of STAT5B in 2 CD4+ T-LGL leukemia patients. Patient 1 had a Q706L mutation at a variant allele frequency (VAF) of 45% in the CD4+CD8− tumor fraction. Patient 2 displayed an S715F mutation (VAF, 36%) in the CD4+ fraction (Figure 1A). Only wild-type (WT) STAT5B was observed in the CD4− fractions, confirming that the mutations were somatic. The third patient with CD4+ T-LGL leukemia did not show any mutations in STAT5B or STAT3 genes, but mutations in members of the protein tyrosine phosphatase family (PTPN14, PTPN23) regulating cell proliferation and tumor suppressor MLL2 were observed (supplemental Table 3).

To study the functional properties of the novel variants, we generated STAT5B expression vectors for WT, Q706L, and S715F mutations and previously described activating N642H mutation.7 The transcriptional activity of the mutants was studied with luciferase reporter assays with and without interferon-α stimulation, and the phosphorylation status was analyzed by western blotting. In HeLa cells,
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