Brief Report

CLINICAL TRIALS AND OBSERVATIONS

Cytokine release syndrome after blinatumomab treatment related to abnormal macrophage activation and ameliorated with cytokine-directed therapy

David T. Teachey,[1,2] Susan R. Rheingold,[1,2] Shannon L. Maude,[1,2] Gerhard Zugmaier,[3] David M. Barrett,[1,2] Alix E. Seif,[1,2] Kim E. Nichols,[1,2] Erica K. Suppa,[4] Michael Kalos,[4] Robert A. Berg,[5,6] Julie C. Fitzgerald,[5,6] Richard Aplenc,[1,2] Lia Gore,[7] and Stephan A. Grupp[1,2]

[1]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [3]Amgen Research (Munich) GmBH, Munich, Germany; [4]Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [5]Division of Critical Care, Children's Hospital of Philadelphia, Philadelphia, PA; [6]Department of Anesthesiology and Critical Care Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; and [7]Department of Pediatrics, University of Colorado Cancer Center, Aurora, CO

Key Points

- Cytokine release syndrome caused by T cell-directed therapies may be driven by abnormal macrophage activation and hemophagocytic syndrome.
- Cytokine-directed therapy can be effective against life-threatening cytokine release syndrome.

Blinatumomab is a CD19/CD3-bispecific T-cell receptor-engaging (BiTE) antibody with efficacy in refractory B-precursor acute lymphoblastic leukemia. Some patients treated with blinatumomab and other T cell-activating therapies develop cytokine release syndrome (CRS). We hypothesized that patients with more severe toxicity may experience abnormal macrophage activation triggered by the release of cytokines by T-cell receptor–activated cytotoxic T cells engaged by BiTE antibodies and leading to hemophagocytic lymphohistiocytosis (HLH). We prospectively monitored a patient during blinatumomab treatment and observed that he developed HLH. He became ill 36 hours into the infusion with fever, respiratory failure, and circulatory collapse. He developed hyperferritinemia, cytopenias, hypofibrinogenemia, and a cytokine profile diagnostic for HLH. The HLH continued to progress after discontinuation of blinatumomab; however, he had rapid improvement after IL-6 receptor-directed therapy with tocilizumab. Patients treated with T cell-activating therapies, including blinatumomab, should be monitored for HLH, and cytokine-directed therapy may be considered in cases of life-threatening CRS. This trial was registered at www.clinicaltrials.gov as #NCT00103285. (*Blood*. 2013;121(26):5154-5157)

Introduction

Blinatumomab (AMG 103) is a bispecific T-cell receptor–engaging (BiTE) single-chain antibody construct designed to link CD19+ B cells with CD3+ T cells, resulting in a cytotoxic T-cell response against CD19+ B leukemia/lymphoma.[1] Blinatumomab is active in adults with relapsed/refractory non-Hodgkin lymphomas and B-precursor acute lymphoblastic leukemia (B-ALL) and is in phase I clinical evaluation in relapsed/refractory pediatric B-ALL (NCT01471782).[2,3] Although blinatumomab has shown remarkable efficacy in early phase B-ALL trials, it also has considerable but manageable toxicity.[3-5] Some patients develop laboratory evidence of transient pronounced cytokine release after starting therapy. Most patients develop transient flu-like symptoms, including fever, chills, and headache, but these can be observed in patients without elevated serum cytokine levels.[4,6]

Interleukin (IL)-6, IL-10, and interferon-γ (INF-γ) are markedly elevated in B-ALL patients receiving blinatumomab.[6] These 3 cytokines are also markedly elevated in children with hemophagocytic lymphohistiocytosis (HLH), also known as macrophage activation syndrome (MAS).[7,8] Several patients receiving CD19-specific chimeric

antigen receptor-modified T cells (CART-19) have developed cytokine release syndrome (CRS), also marked by elevated IL-6, IL-10, and INF-γ.[9] CART-19 is effective in chronic lymphocytic leukemia[10] and is being studied in pediatric B-ALL (NCT01626495). Our group observed that children with B-ALL receiving CART-19 develop CRS with clinical HLH/MAS.[11]

Based on this clinical experience, we hypothesized that blinatumomab-associated CRS may be due to HLH/MAS. To test this hypothesis, we prospectively monitored a patient for HLH/MAS during blinatumomab therapy. He developed fulminant HLH/MAS with multisystem organ failure, which was significantly ameliorated after treatment with IL-6 receptor (IL-6R) inhibitor tocilizumab.

Study design

A 7-year-old male with Trisomy 21 was diagnosed with standard-risk B-ALL 4 years prior to presentation. He was treated according to the Children's Oncology Group protocol AALL0331 (NCT00103285). End-induction minimal
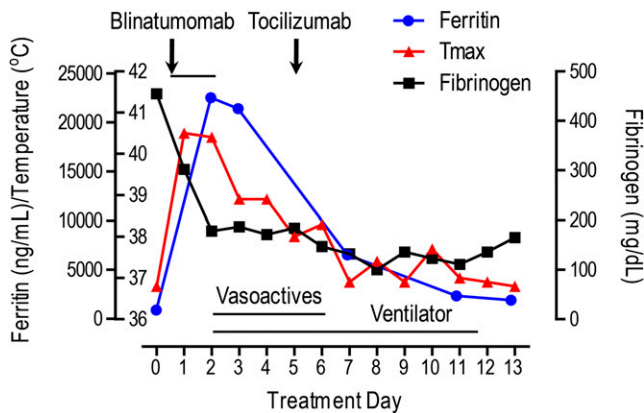
Figure 1. Clinical and laboratory parameters relative to timing of blinatumomab and tocilizumab. Serum ferritin and temperature rapidly rose and serum fibrinogen rapidly fell after starting blinatumomab. Poor systemic perfusion and respiratory failure continued to deteriorate after blinatumomab was stopped; however, these improved significantly after tocilizumab. Initiation of blinatumomab is depicted by arrow and total treatment duration depicted by black line. The blue line represents serum ferritin in ng/mL with circles showing tested values. Red line represents maximum temperature in centigrade (°C) per 24-hour period with triangles demarcating each day. The black line represents fibrinogen with squares depicting tested values. Horizontal axis = treatment days. Time on vasoactive medications (dopamine and epinephrine) is indicated by a black line under "vasoactives" (days 2 to 6). Time on ventilator is indicated by a black line under "ventilator" (days 2 to 11).

residual disease was 5.6%, a poor prognostic finding. Despite high minimal residual disease, he continued on standard-risk B-ALL therapy based on parental concerns of toxicity. Thirty-one months later, he had an isolated bone marrow (BM) relapse. He responded to reinduction chemotherapy, but quickly relapsed, and he was later enrolled on a phase 1 blinatumomab trial. This research was approved by the international review board at the Children's Hospital of Philadelphia and written informed consent was obtained in accordance with the Declaration of Helsinki.

On day 1 of treatment, his white blood cell (WBC) count was 5700/mm³ with 78% blasts. BM evaluation revealed near-complete replacement with lymphoblasts. Six hours after initiating blinatumomab, he developed a high fever (Figure 1) and hyponatremia, with serum sodium of 119 mmol/L (normal range, 138 to 145 mmol/L), down from 142 mmol/L prior to therapy. He developed respiratory distress and hypotension within 36 hours, requiring fluid resuscitation, epinephrine and dopamine infusions, and high-dose dexamethasone. Despite these intensive measures, he remained hypotensive with poor perfusion and severe acidosis (arterial pH 7.18). He required mechanical ventilation for hypoxemic respiratory failure, and received broad-spectrum antimicrobials, although blood cultures remained negative.

To monitor for HLH/MAS prospectively, we obtained a baseline serum ferritin, which was 885 ng/mL (normal range, 10 to 70 ng/mL), consistent with expected mild transfusion-related iron overload. On the day he became critically ill, the ferritin increased to 22 544 ng/mL (Figure 1). His cytopenias worsened as his WBC count dropped to 800/mm³, and his hemoglobin dropped by 2 g/dL. He became co-agulopathic with an international normalized ratio of 2.04, prothrombin time of 23.6 seconds, and partial thromboplastin time of 61.6 seconds. Fibrinogen was normal (455 mg/dL) prior to blinatumomab, then it decreased to a low of 100 mg/dL (normal, 172 to 471 mg/dL), and D-dimer rose from 0.45 ug/mL fibrinogen equivalent units (FEU) to 8.02 ug/mL FEU (normal, 0.1 to 0.6 ug/mL FEU). Triglycerides were normal (80 mg/dL; normal, 32 to 116 mg/dL). He had no hepatosplenomegaly.

Hyperferritinemia >10 000 ng/mL is virtually pathognomonic for HLH/MAS, but it is insufficient to make the diagnosis in isolation.[12,13] The highly elevated ferritin, progressive cytopenias, high fever, and hypofibrinogenemia confirmed the diagnosis of HLH/MAS. Blinatumomab was discontinued approximately 36 hours into the infusion, yet the patient

continued to clinically deteriorate. Based on literature describing elevated IL-6 in patients receiving blinatumomab, we treated the patient with a single 8 mg/kg dose of the IL-6R inhibitor tocilizumab.[6] Within 12 hours, he had a dramatic clinical response. Because of his markedly improved hemodynamic status, we were able to discontinue the epinephrine infusion (in less than 8 hours) and the dopamine (the next day). His perfusion improved and his fever resolved. His respiratory status also quickly improved and he was extubated 7 days post-tocilizumab.

Immediately after tocilizumab treatment, his WBC count transiently increased from 700/mm³ to 4900/mm³, largely comprised of activated lymphocytes on hematopathology review. Ferritin decreased to 6534 ng/mL 48 hours after tocilizumab and 2361 ng/mL by 6 days after tocilizumab. His coagulopathy quickly improved. A cytokine panel obtained 4 days after blinatumomab and 1 day prior to tocilizumab included elevated IL-10 (5338 pg/mL), IL-6 (681 pg/mL), INF-γ (192 pg/mL), and IL-2R (4872 pg/mL), helping to confirm the diagnosis of HLH/MAS (Figure 2).[7,8,14,15] After tocilizumab, his cytokines dramatically improved. *PRF1, STX1, STXBP2,* and *MUNC13-4* mutation testing was normal. NK function testing was not sent because the patient was treated with corticosteroids as part of the blinatumomab protocol therapy and corticosteroids suppress NK function.

Despite the short duration of exposure to blinatumomab, the patient did have a partial response. Peripheral blasts disappeared on day 2 of treatment and a BM sample obtained 18 days after discontinuation of blinatumomab revealed 20% blasts (M2) by morphology.

This patient received blinatumomab at a dose of 30 μg/m²/day on a phase 1 dose-escalation trial. Initial data from this trial show that a second patient experienced the dose-limiting toxicity of CRS at this dose. This trial has established 15 μg/m²/day as the pediatric maximum tolerated dose, and 30 μg/m²/day will not be used moving forward in pediatric B-ALL.[16]

Results and discussion

HLH is a rare condition characterized by inappropriate immune activation and cytokine release that typically presents with fever and splenomegaly in association with hyperferritinemia, coagulopathy, hypertriglyceridemia, and cytopenias.[17] Primary HLH is caused by germline mutations in genes involved in cytolytic granule exocytosis, leading to depressed NK function and allowing macrophage activation to occur spontaneously or with a minimal trigger. Secondary HLH, also known as MAS, is triggered by infection, malignancy, or autoimmune disease. New data suggest that patients with secondary HLH may be predisposed to disease due to the presence of less pronounced defects in cytolytic granule exocytosis or NK function.[17-20] Blinatumomab and other T cell-mediated therapies lead to transient but robust proinflammatory cytokine production, which may trigger HLH/MAS. Our patient did not have an identifiable mutation in a known HLH-predisposing gene.

IL-10, IL-6, and INF-γ are the most highly elevated cytokines in patients who develop cytokine release after blinatumomab treatment[6] and are typically also elevated in HLH/MAS.[7,8] High levels of INF-γ may be expected after blinatumomab, due to release by activated cytotoxic T cells. In contrast, high levels of IL-6 and IL-10 would not be expected with a cytotoxic T-cell response alone and are better explained by HLH/MAS. Because IL-10 is a negative regulator of macrophage function, targeting it may not be prudent in HLH/MAS. High INF-γ may be required for blinatumomab to induce T cell anti-leukemia cytotoxicity, and targeting it could inhibit this activity. IL-6 is principally released by macrophages, causing an inflammatory response. In theory, targeting IL-6 could relieve the toxicity of HLH/MAS without
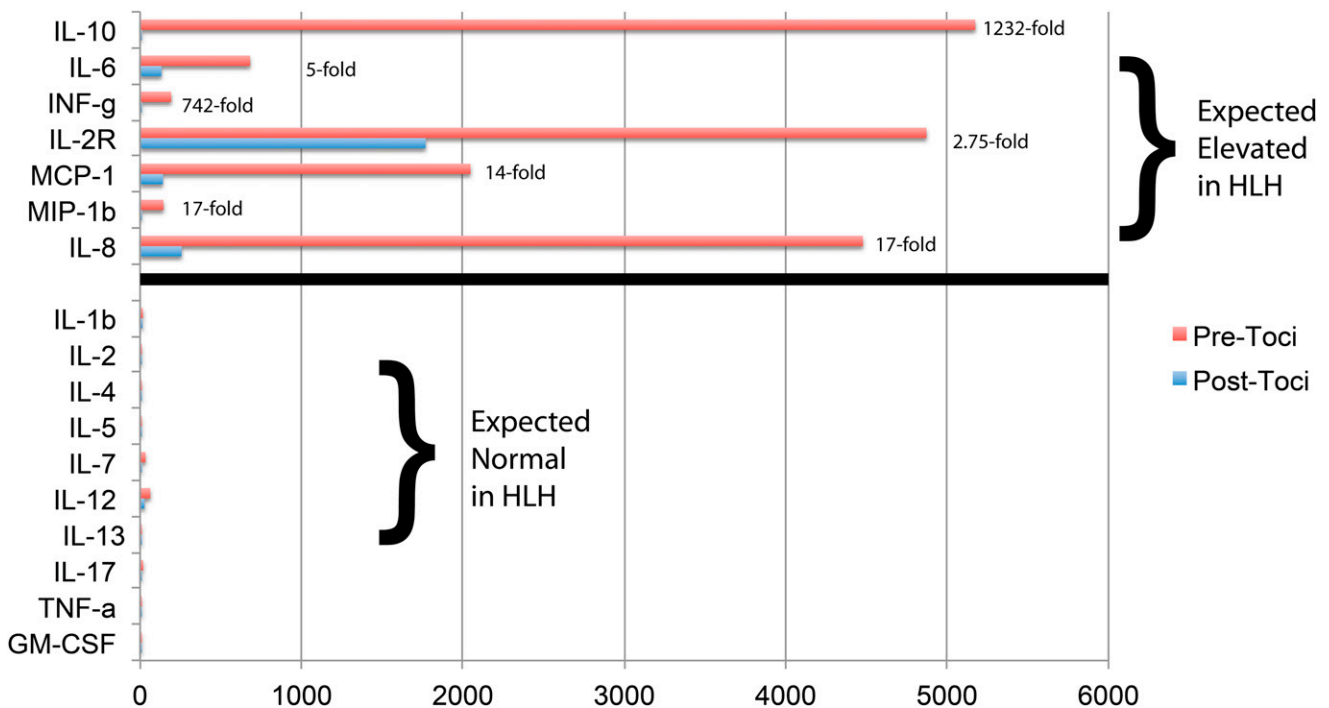
Figure 2. Cytokine and chemokine levels relative to timing of blinatumomab and tocilizumab. Cytokine and chemokine levels were measured 4 days after treatment with blinatumomab and 1 day prior to tocilizumab (toci) (red lines) and repeated 3 days later (2 days after tocilizumab) (blue lines), using Luminex bead array technology and kits purchased from Life Technologies (Invitrogen 30-plex; Carlsbad, CA). Assays were performed according to the manufacturer's protocol with the 9-point standard curve generated using a 3-fold dilution series. Each sample was evaluated in duplicate at 1:3 dilution; calculated percentage (%) of coefficient of variation for the duplicate measures was in most cases less than 5% and always less than 15%. Data were acquired on a FlexMAP-3D and analyzed using XPonent 4.0 software and 5-parameter logistic regression analysis. Standard curve quantification ranges were determined by the 80% to 120% (observed/expected value) range. IL-2R, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, macrophage-inflammatory protein (MIP)1B, and INF-γ were elevated, and IL-1B, IL-4, IL-5, IL-7, IL-12, IL-13, IL-17, tumor necrosis factor (TNF)-a, and granulocyte macrophage–colony-stimulating factor (GM-CSF) were normal. This cytokine prolife is identical to other published work investigating cytokines in children with HLH.[7,8,14,15] IL-2 was normal in this patient. IL-2 can be normal or elevated in HLH; however, IL2R is universally elevated in HLH, as was found in this patient.[7,8,14,15] The top half of the figure (above the black horizontal line) indicate cytokines expected to be elevated in HLH. The bottom half of the figure (below the black horizontal line) indicate cytokines expected to be normal in HLH. X-axis is cytokine level in pg/mL. Numbers beside red/blue line pair represent fold changes in cytokine level pre- and post-tocilizumab. A number of additional cytokines that have not been studied in the published HLH literature were also measured. A complete list of all cytokines tested with absolute values before and after tocilizumab treatment is included, which is described in supplemental Table 1.

impairing T cell-mediated anti-tumor activity. We have also seen HLH/MAS develop in B-ALL patients treated with CART19.[11] We treated some of these patients with tocilizumab and observed that it was effective in reversing the HLH/MAS, but it did not inhibit the activity of the chimeric T cells. It is possible that blinatumomab-induced T-cell proliferation and effector function are preserved after tocilizumab treatment; however, it is also possible that targeting IL-6 could decrease the activity of blinatumomab. Further studies are needed to address this important point. Our patient continued to deteriorate despite dexamethasone and stopping blinatumomab. His improvement immediately after initiating tocilizumab suggests that IL-6R blockade had a significant impact. It is unknown whether earlier tocilizumab use could have ameliorated toxicity and allowed blinatumomab to continue.

In summary, we report a case of blinatumomab-associated HLH/MAS that was reversible using the IL-6R inhibitor tocilizumab. Further studies are needed to determine the prevalence of HLH/MAS after blinatumomab and other T cell-activating therapies and the role of genetic testing for HLH-predisposing genes and NK functional analysis in identifying at-risk patients. Finally, our patient's dramatic improvement with tocilizumab should serve as an impetus for studies of cytokine-directed therapy for patients with HLH/MAS caused by blinatumomab and other cellular immunotherapies.

Authorship

Contribution: D.T.T. proposed the hypothesis and wrote the manuscript; D.T.T., S.A.G., S.R.R., G.Z., and L.G. designed research; D.T.T., S.A.G., S.R.R., G.Z., L.G., S.L.M., K.E.N., and M.K. collected data; D.T.T., S.A.G., S.R.R., G.Z., L.G., S.L.M., K.E.N., A.E.S., R.A., J.C.F., R.A.B., D.M.B., E.K.S., and M.K. analyzed data; E.K.S. performed research; M.K. contributed vital analytical tools and designed research; and S.A.G., S.R.R., G.Z., L.G., S.L.M., A.E.S., R.A., J.C.F., R.A.B., D.M.B., and M.K. helped write the manuscript.

Conflict-of-interest disclosure: G.Z. is employed by and is a shareholder of Amgen Research (Munich) GmBH. The remaining authors declare no competing financial interests.

Correspondence: David T. Teachey, Division of Oncology, Children's Hospital of Philadelphia, 3008 CTRB, 3501 Civic Center Blvd, Philadelphia, PA 19104; e-mail: teacheyd@e-mail.chop.edu.

References

1. Löffler A, Kufer P, Lutterbüse R, et al. A recombinant bispecific single-chain antibody, CD19 x CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. *Blood.* 2000;95(6):2098-2103.

2. Bargou R, Leo E, Zugmaier G, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science.* 2008; 321(5891):974-977.

3. Topp MS, Kufer P, Gökbuget N, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J Clin Oncol.* 2011;29(18):2493-2498.

4. Topp MS, Goekbuget N, Zugmaier G, et al. Anti-CD19 BiTE Blinatumomab induces high complete remission rates in adult patients with relapsed B-precursor ALL: updated results of an on-going phase II trial [614]. *Blood: ASH Annual Meeting Abstracts.* 2011;118:252.

5. Topp MS, Gökbuget N, Zugmaier G, et al. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. *Blood.* 2012; 120(26):5185-5187.

6. Klinger M, Brandl C, Zugmaier G, et al. Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. *Blood.* 2012;119(26):6226-6233.

7. Tang Y, Xu X, Song H, et al. Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome. *Br J Haematol.* 2008;143(1):84-91.

8. Xu XJ, Tang YM, Song H, et al. Diagnostic accuracy of a specific cytokine pattern in hemophagocytic lymphohistiocytosis in children. *J Pediatr.* 2012;160(6):984-990.e1.

9. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med.* 2011;3(95):95ra73.

10. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011; 365(8):725-733.

11. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med.* 2013;368(16):1509-1518.

12. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2008;50(6):1227-1235.

13. Switala JR, Hendricks M, Davidson A. Serum ferritin is a cost-effective laboratory marker for hemophagocytic lymphohistiocytosis in the developing world. *J Pediatr Hematol Oncol.* 2012; 34(3):e89-e92.

14. Olin RL, Nichols KE, Naghashpour M, Wasik M, Shelly B, Stadtmauer EA, Vogl DT. Successful use of the anti-CD25 antibody daclizumab in an adult patient with hemophagocytic lymphohistiocytosis. *Am J Hematol.* 2008;83(9):747-749.

15. Tamura K, Kanazawa T, Tsukada S, Kobayashi T, Kawamura M, Morikawa A. Increased serum monocyte chemoattractant protein-1, macrophage inflammatory protein-1beta, and interleukin-8 concentrations in hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2008; 51(5):662-668.

16. Gore L, Zugmaier G, Handgretinger R, et al. Cytological and molecular remissions with blinatumomab treatment in second or later bone marrow relapse in pediatric acute lymphoblastic leukemia (ALL). *ASCO Meet Abstracts* 2013: 10007.

17. Risma K, Jordan MB. Hemophagocytic lymphohistiocytosis: updates and evolving concepts. *Curr Opin Pediatr.* 2012;24(1):9-15.

18. Zhizhuo H, Junmei X, Yuelin S, Qiang Q, Chunyan L, Zhengde X, Kunling S. Screening the PRF1, UNC13D, STX11, SH2D1A, XIAP, and ITK gene mutations in Chinese children with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2012;58(3):410-414.

19. Zhang K, Jordan MB, Marsh RA, et al. Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. *Blood.* 2011;118(22):5794-5798.

20. Trizzino A, zur Stadt U, Ueda I, et al. Histiocyte Society HLH Study group. Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutations. *J Med Genet.* 2008;45(1):15-21.