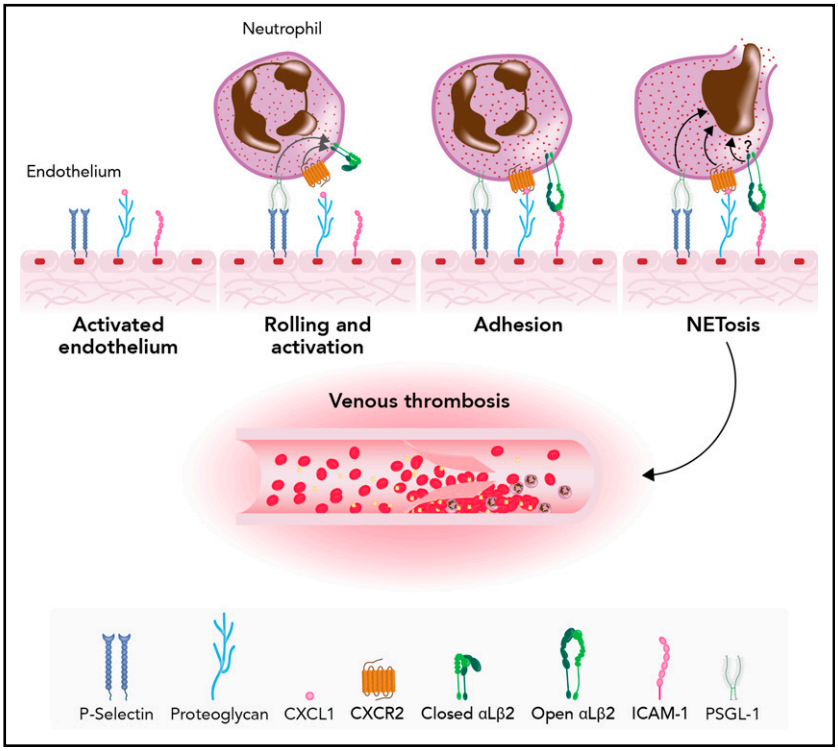

Recruitment of neutrophils and enhancement of venous thrombosis. The activated endothelium expresses P-selectin, ICAM-1, and CXCL1 attached to proteoglycan. Neutrophils are first captured by the activated endothelium by an interaction between P-selectin and PSGL-1, followed by an interaction between CXCL1 and its receptor, CXCR2. Intracellular signaling induced by engagement of PSGL-1 and CXCR2 leads to conformational activation of the αLβ2 (LFA-1) integrin that interacts with ICAM-1 and mediates firm adhesion. NET formation is triggered by signals downstream of PSGL-1 and CXCR2. It is possible that signaling downstream of αLβ2 (LFA-1) also contributes to NET formation. Professional illustration by Somersault18:24.

What is the translational significance of the work? Venous thromboembolism (deep vein thrombosis and pulmonary embolism) is the third leading cause of cardiovascular death in the world. Preventing the recruitment of neutrophils or NET formation should reduce immunothrombosis without affecting hemostasis. There is a long history of targeting P-selectin as a novel antithrombotic strategy. P-selectin is expressed on the surface of activated endothelial cells and platelets. In 1992, it was shown that inhibition of P-selectin reduced leukocyte accumulation and fibrin deposition in an arteriovenous shunt model in baboons.[8] This work was followed by a number of elegant studies by the Wakefield group showing that inhibition of P-selectin reduced venous thrombosis in animal models, including a baboon model.[9] These studies indicate that targeting P-selectin reduces thrombosis and inflammation without increasing bleeding. The humanized monoclonal anti–P-selectin antibody crizanlizumab blocks PSGL-1 interaction with P-selectin and is currently being evaluated for the prevention of vaso-occlusive crises in patients with sickle cell disease.[10] It would be very interesting to assess if crizanlizumab or other P-selectin inhibitors reduce venous thrombosis in humans.

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REFERENCES

1. Yago T, Liu Z, Ahamed J, McEver RP. Cooperative PSGL-1 and CXCR2 signaling in neutrophils promotes deep vein thrombosis in mice. *Blood*. 2018;132(13):1426-1437.
2. Antoniak S. The coagulation system in host defense. *Res Pract Thromb Haemost*. 2018; 2(3):549-557.
3. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol*. 2013;13(1):34-45.
4. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532-1535.
5. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014;123(18):2768-2776.
6. Budnik I, Brill A. Immune factors in deep vein thrombosis initiation. *Trends Immunol*. 2018; 39(8):610-623.
7. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007;7(9):678-689.
8. Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. *Nature*. 1992;359(6398):848-851.
9. Wakefield TW, Myers DD, Henke PK. Role of selectins and fibrinolysis in VTE. *Thromb Res*. 2009;123(suppl 4):S35-S40
10. Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med*. 2017; 376(5):429-439.

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TRANSPLANTATION

Comment on Allen et al, page 1438

Finding "intermediate" ground in transplant and HLH

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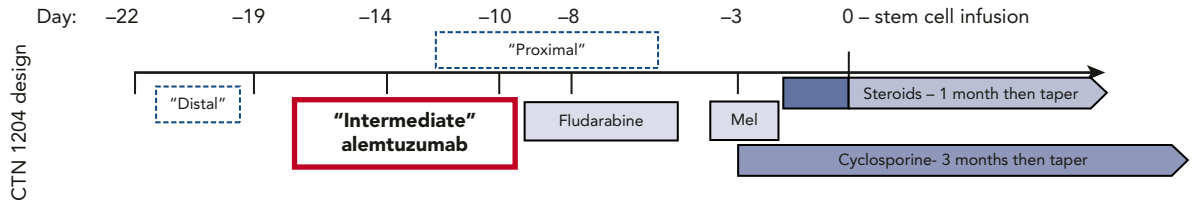
The Bone Marrow Transplant Clinical Trials Network (BMT CTN) study 1204 of stem cell transplantation for hemophagocytic lymphohistiocytosis (HLH) and primary immunodeficiencies (PID) presented in this issue of *Blood* by Allen et al emphasizes how far treatment and outcomes in these rare diseases have come but also how important multicenter trials are to find a "sweet spot" in the design of curative regimens.[1]

Large-scale global observational studies in pediatric HLH (familial, acquired, and associated with PIDs) established treatment algorithms containing etoposide and steroids and a curative role for hematopoietic stem cell transplantation (HSCT) in 1994. Specifically, the HLH-1994 study experience in 113 children demonstrated

A

Treatment approach advance	Intervention	Outcome
Role for allogeneic HSCT in HLH[2-4]	Etoposide and dexamethasone followed by myeloablative HSCT	3-year OS entire population 55%, 3-year OS post-HSCT 62%[2] **Caveat**: significant organ toxicity
RIC[5,6]	Intensity reduction – multiple regimens	Increased survival (80-100%), decreased toxicity **Caveats**: significant nonengraftment, mixed chimerism especially in RIC (65%)
Alemtuzumab pre-HSCT[8-10]	Proximal: days –12 to –9 to days –9 to –5 Distal: days –22 to –19 Intermediate: days –14 to –10	Distal vs proximal - less mixed chimerism Intermediate – mixed chimerism 31%, second cell therapy 14%
CTN 1204 – intermediate alemtuzumab dosing[1]	Fludarabine/melphalan RIC backbone in HLH/PID Alemtuzumab – intermediate: days - 14 to -10, 1 mg/kg total	Established benchmarks in multicenter study: 1-year OS 80%, 18-month OS 67% 1-year OS without second cell therapy 39.1% **Caveats**: infection rates, second cell therapy, TRM, GVHD

B



(A) Selected advances in role of stem cell transplantation in hemophagocytic lymphohistiocytosis and primary immunodeficiencies. (B) Schema of BMT CTN study 1204 transplant regimen and timing of alemtuzumab dosing (vs prior studies). Mel, melphalan; OS, overall survival; RIC, reduced-intensity conditioning; TRM, transplant-related mortality.

3-year survival of 55%; in the 58% of patients who underwent HSCT, survival was 62%. This represented a huge advance in treating what was previously an almost universally fatal disease of immune activation and dysregulation.[2] Ten-year follow-up from HLH-1994 (n = 249), data from the HLH-2004 study involving up-front calcineurin inhibition (n = 369), and results from smaller cohorts demonstrated 5-year survival rates of 54% to 61%; again, those who were transplanted (~50%) had better survival of 50% to 70%.[3,4] Up to 20% of subjects may survive without transplantation, but HSCT is considered a necessary intervention for most individuals, especially those with familial HLH (see figure panel A).

Although encouraging, these cohorts highlighted an early mortality rate of close to 30% following HSCT with primarily myeloablative conditioning. Causes of transplant-related mortality included liver toxicity (particularly veno-occlusive disease), viral infections, respiratory and other organ failure, and graft-versus-host disease (GVHD).[3,4] Introduction of RIC to reduce complications boosted survival rates to 75% to 100%.[5] One retrospective analysis demonstrated 3-year survival of 43% following myeloablative conditioning (n = 14) vs 92% following RIC (n = 26).[6] The most common RIC regimens used fludarabine, melphalan, and either alemtuzumab or antithymocyte globulin.

Unfortunately, reduction of toxicities with RIC regimens came with increased incidence of mixed chimerism (eg, >5% residual recipient or <95% donor cells) following HSCT: 65% after RIC vs 18% after myeloablative conditioning in 1 study.[6] There are no data to correlate a specific threshold of total leukocyte or T-cell chimerism with optimal survival. However, multiple studies have shown >20% to 30% chimerism of donor cells is associated with, although not necessary for, sustained remission of HLH.[7] This led to recent practice patterns wherein >80% of patients with mixed chimerism receive a second transplant, $CD34^+$ selected stem cell boost, and/or T-cell containing donor lymphocyte infusion. In some trials, the majority of patients received at least 1 additional cellular therapy after RIC HSCT.[7]

Alemtuzumab has historically been used during HSCT conditioning to achieve in vivo T-cell depletion of the graft and decrease GVHD. In HLH, alemtuzumab alone has been shown to be an effective immunomodulatory salvage therapy in those failing etoposide and steroids.[8] Therefore, utilization of alemtuzumab with HSCT for HLH and other states of immune dysregulation such as PID might accomplish several goals: (1) enhance primary HLH control; (2) deplete recipient T cells to improve engraftment; (3) deplete recipient antigen-presenting cells and donor T cells to decrease inflammation and GVHD. However, donor T-cell depletion might also be expected to impede engraftment and achievement of full donor chimerism. These hypotheses drove investigation of "proximal" alemtuzumab timing (days −12 to −8 before infusion) with potentially more effect on the graft vs "distal" timing (days −22 to −19) with perhaps more impact on recipient immune cells. Two single-center trials demonstrated less mixed chimerism with "distal" dosing.[6,9] "Intermediate" alemtuzumab at days −14 to −10 was found to further reduce rates of mixed chimerism: subsequent cell therapy rate of 14% vs 53% after proximal and 38% after distal dosing.[10]

The BMT CTN study 1204 reported here is a US-based, prospective, phase 2 trial conducted at 22 sites and involving 34 children and young adults with HLH and 12 with PID (median age, 2.3 years [range, 5 months to 28 years]) who underwent a RIC fludarabine and melphalan-based

HSCT with bone marrow from fully HLA-matched related or 7 to 8/8 HLA-matched unrelated donors.[1] "Intermediate" dosing of alemtuzumab was used, along with cyclosporine and methylprednisolone as GVHD prophylaxis (see figure panel B). Findings were 1-year survival of 80% and 18-month survival of 67%. More than 50% of patients had graft failure or received another cellular intervention. A slightly higher than expected transplant-related mortality rate of 13% was observed, resulting from bacterial and viral infections, organ failure, and GVHD. These data provide benchmarks for further trials of HSCT in HLH and PID. Immediate next steps include optimization of alemtuzumab pharmacokinetics perhaps based on recipient lymphocyte counts and consideration of alternative GVHD prophylaxis approaches. Of note, the level of residual recipient chimerism for which additional action was taken was highly variable, and those patients who did reactivate HLH all had donor T-cell chimerism >37%. Thus, the exact relevance and priority of mixed chimerism as an end point and rational thresholds for further cell therapy after HSCT in HLH deserve further study.

Although survival was slightly lower and the incidence of mixed chimerism was higher than expected after prior single-center studies, the multicenter BMT CTN 1204 trial proves, first, how much progress has been made in survival for patients with HLH and PID and, second, how sequential single-institution and cooperative group trials can rationally address discrete questions even in very rare diseases. In addition to highlighting specific research questions related to alemtuzumab timing, immune reconstitution, and HSCT regimen design, this study lays the basis for trials of alternative haploidentical and umbilical cord donors, of novel anticytokine therapies and antibody-based conditioning, and for genetic modification of hematopoietic stem cells or T cells in hereditary diseases such as HLH and PID. The field will not rest on this "intermediate" ground!

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REFERENCES

1. Allen CE, Marsh R, Dawson P, et al. Reduced-intensity conditioning for hematopoietic cell transplant for HLH and primary immune deficiencies. *Blood*. 2018;132(13):1438-1451.
2. Henter J-I, Samuelsson-Horne A, Aricò M, et al; Histocyte Society. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood*. 2002;100(7):2367-2373.
3. Trottestam H, Horne A, Aricò M, et al; Histiocyte Society. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood*. 2011;118(17):4577-4584.
4. Bergsten E, Horne A, Aricó M, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. *Blood*. 2017;130(25):2728-2738.
5. Cooper N, Rao K, Goulden N, Webb D, Amrolia P, Veys P. The use of reduced-intensity stem cell transplantation in haemophagocytic lymphohistiocytosis and Langerhans cell histiocytosis. *Bone Marrow Transplant*. 2008;42(suppl 2):S47-S50.
6. Marsh RA, Vaughn G, Kim M-O, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood*. 2010;116(26):5824-5831.
7. Hartz B, Marsh R, Rao K, et al. The minimum required level of donor chimerism in hereditary hemophagocytic lymphohistiocytosis. *Blood*. 2016;127(25):3281-3290.
8. Marsh RA, Allen CE, McClain KL, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. *Pediatr Blood Cancer*. 2013;60(1):101-109.
9. Oshrine BR, Olson TS, Bunin N. Mixed chimerism and graft loss in pediatric recipients of an alemtuzumab-based reduced-intensity conditioning regimen for non-malignant disease. *Pediatr Blood Cancer*. 2014;61(10):1852-1859.
10. Marsh RA, Kim M-O, Liu C, et al. An intermediate alemtuzumab schedule reduces the incidence of mixed chimerism following reduced-intensity conditioning hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Biol Blood Marrow Transplant*. 2013;19(11):1625-1631.

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