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PROGNOSTIC SIGNIFICANCE OF PRETREATMENT SERUM CYTOKINES IN CLASSICAL HODGKIN LYMPHOMA

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Abstract

Purpose: While the International Prognostic Score (IPS) is the gold standard for risk-stratifying patients with classical Hodgkin lymphoma (cHL), these criteria do not accurately predict outcome. As cytokines are critically involved in driving cHL, we tested whether pretreatment serum cytokine levels could provide additional prognostic information.

Experimental Design: Thirty cytokines were measured in pretreatment serum from 140 cHL patients and compared with 50 non-lymphoma controls. Patients were followed for event-free and overall survival, and Cox proportional hazards regression models were used to assess the association of individual cytokines and the cytokine profiles with outcome via unadjusted and IPS-adjusted hazard ratios (HR).

Results: Twelve cytokines (EGF, FGFb, GCSF, HGF, IL-6, IL-8, IL-12, IL-2R, IP-10, MIG, TNF α and VEGF) were significantly ($p < 0.05$) higher in cHL patients than controls; elevated levels of HGF, IL-6, IL-2R, IP-10 and MIG were all associated with poorer event-free survival (EFS). Only IL-2R ($p = 0.002$) and IL-6 ($p < 0.001$) were independently prognostic. Patients with increased IL-6 and IL-2R had a significantly higher risk of early relapse and death, a finding that remained significant even after IPS-based risk stratification. While elevated IL-6 and IL-2R correlated with the IPS, sCD30 and TARC levels, the 2-cytokine model remained independently predictive of prognosis.

Conclusions: Elevated pretreatment serum cytokines are associated with increased disease relapse and inferior survival in cHL. Thus, the pretreatment cytokine profile, particularly serum levels of IL-6 and IL-2R, may be used to identify cHL patients at high risk for early disease relapse.

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Authorship contributions

S.M.A. and A.J.N. designed the experiments and analyzed and interpreted the data. P.R.M., L.S.H. and M.J.M. analyzed data and drafted the paper. S.L.S., T.M.H., B.K.L. and J.R.C. collected data and provided patient specimens.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Introduction

Classical Hodgkin lymphoma (cHL) is a malignant disorder of lymphoproliferative origin hallmarked by the presence of Reed-Sternberg (RS) cells and an extensive inflammatory cell infiltrate.(1) While most patients diagnosed with cHL will be cured with the use of combination chemotherapy regimens and radiation, 10-20% of patients will experience progression of the disease.(2) The International Prognostic Factors Project Score (IPS) is the gold standard used to risk-stratify patients with advanced-stage cHL, but the IPS is not able to identify patients in whom treatment is likely to fail.(3) More accurate predictions of patient outcome in cHL are needed and may be realized through the identification of novel biomarkers.

While RS cells are morphologically characteristic of cHL, reactive cells within the tumor microenvironment greatly outnumber the malignant cell population and play an important role in driving the progression of this malignancy.(4) Lymphocytes, macrophages, eosinophils and mast cells, amongst other reactive cell types, all interact with malignant cHL cells, mainly via cytokine and chemokine crosstalk, thereby promoting malignant cell growth and survival while increasing the proliferation of RS cells.(5) Conversely, cytokines secreted by the RS cells themselves are thought to affect the recruitment and biological activity of non-malignant cells in the tumor microenvironment, leading to an abnormal immune response and heightened inflammation.

It is therefore not surprising that in addition to certain clinical factors and other soluble markers such as soluble CD30 (sCD30), transferrin, and β -2 microglobulin, serum levels of many cytokines have been observed to correlate with prognosis in cHL. Elevated levels of CC thymus and activation-related chemokine (TARC), interleukin 10 (IL-10), interleukin 13 (IL-13) and CCL17, have all been associated with poorer outcomes in cHL patients.(6-9) Additionally, interleukin 6 (IL-6), which is highly secreted by both HRS cells and surrounding reactive cells, is also believed to play a critical pathobiological role in cHL.(10) High serum levels of this cytokine have been detected in patients with advanced cHL regardless of stage, with levels decreasing significantly in response to treatment.(11)

As cytokine- and chemokine-mediated crosstalk between malignant cells and reactive cells in the tumor microenvironment is known to regulate the pathobiology of cHL, our goal here was to determine whether pretreatment serum cytokine levels could be predictive of disease prognosis in cHL patients. To this end, a panel of thirty selected cytokines and other immune markers were measured in pretreatment serum specimens obtained from patients with cHL and compared with serum levels in healthy control subjects. We have identified IL-6 and IL-2R to be significantly associated with clinical outcome, suggesting that the pretreatment cytokine profile may be useful in identifying cHL patients at high risk for early disease relapse. Additionally, these data indicate that specific targeting of cytokine- and chemokine-mediated interactions in the tumor microenvironment may provide a novel therapeutic strategy for improving treatment outcomes in patients with cHL.

Methods

Study population

Patients newly diagnosed with cHL were prospectively enrolled into the University of Iowa/ Mayo Clinic SPORE Molecular Epidemiology Resource (MER) after providing written informed consent. This research was reviewed and approved by the Human Subjects Institutional Review Boards at both the University of Iowa and Mayo Clinic. Clinical data were abstracted using a standard protocol and all patients were followed systematically for event-free (progression, retreatment, or death due to any cause) and overall survival (EFS

and OS, respectively). Clinic-based controls were enrolled through Mayo Clinic Department of Medicine following a prescheduled general medical examination. Eligibility requirements for the reference population included age > 20 years, resident of Minnesota, Iowa or Wisconsin and no prior diagnosis of lymphoma, leukemia or HIV infection.

Measurement of serum cytokines

Thirty serum cytokines were measured from pretreatment blood draws using a multiplex ELISA (Upstate Cell Signaling Solutions, Lake Placid, NY) Plates were read using the Luminex-200 system Version 1.7 (Luminex, Austin, TX), and data analyzed using the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA). The following cytokines were quantified: epidermal growth factor (EGF), eotaxin, basic fibroblast growth factor (FGFb), granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony stimulating factor (GCSF), hepatocyte growth factor (HGF), interferon alpha (IFN α), interferon gamma (IFN γ), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IL-12p40, soluble interleukin-2 receptor (sIL-2R), interleukin-1 receptor antagonist (IL-1RA), interleukin-1 beta (IL-1b), monocyte chemotactic protein 1 (MCP-1), inducible protein 10 (IP-10)/CXCL10, monokine induced by interferon γ /CXCL9 (MIG), MIP-1a (CCL3), MIP-1b (CCL4), regulated on activation, normal T-cell expressed and secreted (RANTES)/CCL5, TNF α (tumor necrosis factor alpha), and VEGF (vascular endothelial growth factor). Interassay variation was assessed by inclusion of an internal control serum on all assays plated; the CV for the internal control log₂ cytokine values across plates ranged from 0.3% (RANTES) to 12.2% (IL-6). To further control for any variation in the cytokine results due to lot-to-lot variability in the ELISA plates used, separate control specimens were used for each of the cohorts. Individual ELISAs were performed according to the manufacturer's protocols to quantify serum levels of sCD30 and CCL17/TARC (sCD30, eBioscience, San Diego, CA; CCL17/TARC, R&D Systems, Minneapolis, MN).

Immunohistochemistry

Immunohistochemistry was performed to assess the intratumoral expression of IL-6 (sc-130326, Santa Cruz Biotechnology, Santa Cruz, CA), IL-2R (NCL-CD25-305, Leica Microsystems, Buffalo Grove, IL), CD30 (M0751, DAKO Cytomation, Carpinteria, CA) and TARC (sc-12271, Santa Cruz Biotechnology, Santa Cruz, CA). Formalin fixed paraffin embedded tissue from consenting patients with cHL were cut at 4 μ m and dried over night at 37°C. All slides were deparaffinized with two changes of xylene and rehydrated through alcohol series. The tissue was treated with methanol/hydrogen peroxide to quench endogenous peroxidase activity. After rinsing with water, the tissue was subjected to antigen retrieval with heated 1mM EDTA, pH 8.0, and rinsed well with Tris-buffered saline. Non-specific binding sites were blocked by incubation with 10% non-immune goat serum. Primary antibodies were added to tissue sections, followed by appropriate biotinylated secondary antibodies and then with HRP-streptavidin. Specific cells were visualized with 3, 3'-diaminobenzidine and counterstained with hematoxylin. After mounting, slides were visualized on an Olympus Provis AX70 light microscope.

To create immunohistochemical overlays of IL-6 and sIL-2R staining together with CD30 and CD25, the SIMPLE (Sequential Immunoperoxidase Labeling and Erasing) method with AEC was used as described previously.⁽¹²⁾ In brief, sections were first stained for IL-6, and, after detection with DAKO Advance System and AEC, the tissue was destained in 95% alcohol to remove AEC, and the antibody stripped with glycine/SDS (pH 2) at 50°C for 30 minutes. The sections were then stained with IL-2R followed by CD30 using the same detection system. Digital snapshots of all sections were captured for each antibody, and multicolor, composite images were made by overlaying each section using Adobe Photoshop CS2 with separate colors assigned to individual stains.

Statistical analyses

The primary analysis was performed to determine the relationship between cytokine levels and either event-free survival (EFS) or overall survival (OS) in patients with cHL. Each of the thirty cytokines measured was evaluated as a normal vs. elevated dichotomous variable, with the upper limit of normal for each cytokine defined as the 95th percentile from the distribution of the control population. Correlations between cytokines were assessed using Spearman correlation coefficients. EFS was defined as the time from initial diagnosis to disease progression, retreatment or death due to any cause. Patients without an event or death were censored at the time of their last known follow-up. Cox proportional hazards regression models were used to assess the association of individual cytokines and the cytokine profiles with outcome via unadjusted and IPS-adjusted hazard ratios (HR). Kaplan-Meier curves were used to graphically display the association of cytokines with outcome. Analyses were performed using SAS v9.1.3.

Results

Study population

One hundred and forty patients with cHL were enrolled in this study with a median age of forty years (Table 1). Seventy-three of the 140 patients were male. At their initial diagnosis, the majority of patients had limited stage disease (55%), and 58% were low or low-intermediate risk by the IPS. For treatment, 86% of patients received ABVD chemotherapy, with the remainder received the Stanford V regimen (9%), BEACOPP (4%) or MOPP chemotherapy (2%). At a median follow-up of 47 months (range 0-87), 18% of patients had an event and 11% had died, about a quarter of deaths were due to other causes.

Cytokines in cHL

Of the thirty cytokines initially measured, the serum levels of twelve cytokines including EGF, FGFb, GCSF, HGF, IL-6, IL-8, IL-12, IL-2R, IP-10, MIG, TNF α and VEGF, were significantly higher in cHL patients than non-lymphoma controls (Table 2). Of these, sIL-2R, IP-10, MIG, IL-6 and HGF were significantly associated with an increased sedimentation rate, B-symptoms, advanced stage and splenic involvement, and elevated IL-8 levels were associated with bulky disease. There was also a modest correlation between IL-6 and platelet count ($p=0.033$). Furthermore, cytokine elevation was associated with EFS at $p<0.05$ in 5 cytokines, including HGF (HR=3.57, $p=0.0016$, CI=1.60-7.98), IL-6 (HR=6.15, $p=6.2\times 10^{-6}$, CI=2.8-13.53), IL-2R (HR=4.08, $p=0.0049$, CI=1.53-10.89), IP-10 (HR=3.23, $p=0.0041$, CI=1.45-7.20) and MIG (HR=2.47, $p=0.027$, CI=1.11-5.50) (Table 3). Only IL-6 ($p<0.001$) and sIL-2R ($p=0.002$) were independently prognostic for EFS (Figures 1A, 1B). To account for multiple cytokine levels being compared, a Bonferroni correction for cytokine association with EFS of $(0.05/30 = 0.0016)$ for all 30 measured cytokines or $(0.05/12 = 0.0042)$ for 12 elevated cytokines, could be applied. However given the known biologic correlation between cytokines, and since these are not truly independent variables, we report the raw p -values for the associations and further evaluated cytokines associated with EFS at a value of $p<0.05$.

Of 59 patients with normal levels of IL-6 and IL-2R, only 2 (3.4%) had events within 12 months and 4 had events any time. Of 60 patients with only one elevated cytokine level, 4 (6.7%) had events within 12 months and 9 had events any time. So outcome was excellent amongst patients with either normal levels of both cytokines or only one elevated cytokine. In contrast, of 20 patients with both IL-6 and IL-2R elevated, 12 (60%) had events within 12 months and 12 had events any time. Compared with patients in whom levels of IL-6 and sIL-2R were within normal limits and patients in whom only one of these cytokines was elevated, patients with high levels of both IL-6 and IL-2R had significantly shorter EFS and

OS (EFS HR=8.90, 4.03-19.67, $p<0.0001$), Figures 1C, 1D). This 2-cytokine score remained significant even after adjusting for stage using Cox models for individual cytokines (EFS HR=7.77, CI=3.44-17.58, $p<0.0001$).

The analyses were then repeated after risk-stratifying patients based on IPS. While levels of IL-6 and sIL-2R both correlated with the IPS (both $p<0.002$), the 2-cytokine model remained independently predictive of prognosis. When only low-risk patients were considered (IPS 0-3), the 2-cytokine model remained predictive of early relapse (Figure 2A). Consistent trends were observed in the high-risk patient cohort as well (IPS-4-7, Figure 2B).

Correlation with other soluble factors

As increased levels of the sCD30 and the chemokine TARC have both been previously associated with poorer outcomes in cHL, we were interested in determining whether serum levels of either IL-2R or IL-6 correlated with these known biomarkers. (6, 13) sCD30 and TARC levels were subsequently measured in the serum of 119 and 120 patients, respectively, of the original 140 cHL patient cohort on whose serum specimens the initial 30-plex ELISA was performed. While both sCD30 and TARC levels were significantly higher in the cHL cohort as compared to the reference population, only elevated sCD30 was significantly associated with EFS (HR=3.99, $p=0.012$). IL-2R levels strongly correlated with sCD30 ($r=0.82$, $p<0.00001$) and with TARC ($r=0.36$, $p<0.0001$). Similarly, serum levels of IL-6 also correlated with both sCD30 and TARC ($r=0.46$, $p<0.0001$ and $r=0.24$, $p=0.004$, respectively). In modeling the effect on EFS, elevated sIL-2R was more informative than sCD30, and sCD30 was not significant ($p=0.63$) when included in a Cox model for EFS with sIL-2R and IL-6. A Cox model with sIL-2R and IL-6 had better concordance ($c=0.763$) and fit (log likelihood=187.2) than a model with sCD30 and IL-6 ($c=0.755$, log likelihood=189.8). Levels of IL-2R and IL-6 remained individually associated with outcome after adjusting for TARC and sCD30 (both $p<0.0001$) and the two-cytokine sum variable also remained prognostic ($p=0.0002$) after adjustment.

Source of elevated cytokines in cHL

We next evaluated whether the increased serum cytokine levels were due to increased expression in the lymph nodes. To determine the cellular source of the elevated cytokines in cHL, immunohistochemistry was performed on lymph node sections obtained from 22 patients. Staining of CD30, IL-6, and IL-2R was observed in the diagnostic lymph node biopsy specimens (Figure 3A-C) and was mainly localized to RS cells. Costaining staining of cHL tumor sections with IL-2R and IL-6 followed by CD30 revealed staining of IL-6 in sCD30⁺ HRS cells. IL-2R also costained with CD30, but the overall staining pattern was more diffuse than that observed with IL-6 (Figure 3D and inset) suggesting that IL-2R may be expressed on other cells aside from the RS cells.

Discussion

Numerous studies suggest that cHL is a malignancy defined by a unique cytokine profile. Cytokines and chemokines present within cHL tumors contribute not only to the growth and survival of HRS cells, but also to the maintenance of an immunosuppressive and inflammatory environment through the recruitment of reactive cells to cHL tissues. (14-17) Both HRS cells and the reactive cells within the tumor microenvironment are responsible for the overproduction of specific cytokines, and elevated serum levels of many of these soluble factors have been associated with tumor bulk, stage and aggressiveness in patients with cHL. Because of this, we investigated whether the pretreatment serum concentrations of a panel of cytokines could be used as reliable prognostic markers in patients recently diagnosed with cHL.

Of the thirty cytokines initially analyzed, five were significantly associated with EFS, two of which, IL-6 and IL-2R, remained independently prognostic of clinical outcome even after adjusting for IPS score. As a follow-up to this analysis, we also measured levels of sCD30 and TARC in 119 and 120, respectively, of the initial 140 cHL patients enrolled. As has been reported previously, levels of both sCD30 and TARC were significantly higher in the cHL cohort as compared to non-lymphoma controls.(6, 8, 13) However, only sCD30 was associated with EFS in our study. Both IL-2R and IL-6 individually correlated with serum levels of sCD30, yet the two-cytokine model of IL-2R and IL-6 remained independently predictive of clinical outcome even after adjusting for sCD30 levels. Furthermore, immunohistochemistry demonstrated costaining of both sIL-2R and IL-6 with sCD30 in lymph node samples obtained from cHL patients. Therefore, not only are these two cytokines tumor-derived biomarkers, but using pre-treatment serum levels of IL-6 and IL-2R to risk-stratify cHL patients may prove to be more clinically relevant than using IPS score or other prognostic biomarkers. In fact, while patients with IPS scores of 0-3 are typically thought to respond well to therapy, with a recent meta-analysis reporting a freedom from progression rate of 81% for this population, our study indicates that patients with the same IPS scores but elevated levels of IL-6 and IL-2R have an EFS of only 54%.(18) Similarly, the 2-cytokine model identifies a cohort of patients with IPS scores of 4-7 predicted to have an EFS of only 15% compared to the 65% for this patient subset predicted based on IPS score alone. Our data suggest that using the 2-cytokine model to identify patients at higher risk of disease progression and, subsequently, treating this patient population more aggressively, may improve overall outcomes.

IL-6 is known to have important immunomodulatory functions influencing the growth and differentiation of both B and T cells, and our study is not the first to report a pathogenic role for this pleiotropic cytokine in cHL. As has been observed with many malignancies, including multiple myeloma, non-Hodgkin lymphomas, renal cancer and ovarian cancer, serum levels of IL-6 are frequently detected at high levels in cHL patients at the time of initial diagnosis with levels normalizing upon remission.(19) Additionally, while IL-6 has been shown to be produced by lymphocytes, macrophages, and fibroblasts within the cHL tumor microenvironment, IL-6 production by the malignant HRS cells, as determined by immunohistochemical staining, has been observed to correlate with the presence of 'B' symptoms and the likelihood of achieving a complete response to therapy.(7) (20, 21) While the specific mechanism by which IL-6 regulates cHL biology and produces a more aggressive tumor is still unclear, our data suggest that elevated pretreatment serum levels of IL-6, most likely derived from HRS cells, significantly correlate with early relapse in cHL.

Similarly, IL-2R molecules have also been detected previously in the supernatant of cHL-derived cell lines as well as in the serum of patients with cHL. High serum IL-2R has been associated with the presence of 'B' symptoms, more advanced stage disease, and significantly lower rates of remission.(22) Yet, the biological mechanism by which serum IL-2R drives the progression of cHL is not known. However, clues may be gleaned from studies of follicular lymphoma, where high levels of soluble IL-2R are also associated with a poor prognosis. In vitro work from our laboratory has revealed that soluble IL-2R, instead of binding IL-2 and preventing its activity, actually facilitates IL-2 signaling via STAT5, inducing T cell proliferation and enhancing Foxp3 expression on CD4+ T cells in the tumor microenvironment.(23) This ultimately leads to a skewing of intratumoral T cells towards a more regulatory phenotype and inhibits the function of CD8+ T cells, suggesting that in vivo, interactions between IL-2R and IL-2 may suppress antitumor immunity by preventing the activity of intratumoral effector T cells. We hypothesize that a similar phenomenon may be occurring within the cHL tumor microenvironment as well, and, that instead of binding and inhibiting IL-2 function, the elevated serum levels of IL-2R detected in many cHL

patients may actually serve to further limit the host immune response, potentially leading to more aggressive tumors and poorer outcomes.

In conclusion, we have observed a significant association between elevated pretreatment serum levels of both IL-2R and IL-6 and clinical outcome in cHL patients. These two cytokines remain predictive of patient response even after adjusting for sCD30 levels, a known prognostic biomarker in cHL. This suggests that the pretreatment cytokine profile may identify patients at high risk for early disease relapse, independently of IPS, and who may benefit from more aggressive therapy. Lastly, further investigations into the therapeutic targeting of crosstalk between the cytokine-secreting HRS cells and reactive cells in the cHL tumor microenvironment may yield more effective treatment options for patients with this malignancy.

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Translational Relevance

This study has investigated the clinical relevance of using pretreatment serum cytokine levels as prognostic biomarkers in patients diagnosed with classical Hodgkin lymphoma. A predictive 2-cytokine model has been identified whereby elevated pretreatment serum levels of both IL-2R and IL-6 have been significantly correlated with poorer outcomes in this patient population. To date, the International Prognostic Score (IPS) has been used as the gold-standard to risk-stratify patients with cHL, but our data indicate that patients with elevated IL-2R and IL-6 have significantly lower response rates following treatment than predicted by IPS score alone. These data not only suggest that IL-2R and IL-6 may contribute to the progression of cHL and may serve as potential therapeutic targets, but that pretreatment cytokine screening may be useful clinically to identify cHL patients at high risk for early relapse who may benefit from more aggressive therapy.

Figure 1. Outcome of cHL patients by IL-2R and IL-6 serum levels

(A) EFS of all patients by serum IL-6 levels, (B) EFS of all patients by serum IL-2R levels, (C) EFS of all patients by 2-cytokine model (IL-6 and IL-2R), (D) OS of all patients by 2-cytokine model (IL-6 and IL-2R).

Figure 2. Outcome of cHL patients by IL-2R and IL-6 serum levels after risk-stratification
 (A) EFS in low risk (IPS 0-3) patients by 2-cytokine model (IL-6 and IL-2R), (B) EFS in high risk (IPS 4-7) patients by 2-cytokine model (IL-6 and IL-2R).

Figure 3. Detection of intratumoral IL-6, IL-2R and sCD30

Immunohistochemical staining of (A) CD30, (B) IL-6 and (C) IL-2R in lymph nodes sections obtained from 22 consenting patients with cHL was performed as described in “Methods”. Slides were visualized on an Olympus Provis AX70 light microscope, and images shown are original magnification X400. (D) Co-staining of IL-2R and IL-6 with HRS cells (CD30) in cHL lymph nodes. Sections were first stained for IL-6 (blue), stripped, and restained for IL-2R (yellow) and CD30 (red). Digital images from each layer were then overlaid. A representative multicolor composite image is shown.

Table 1

Patient characteristics

	cHL Patients	
	<i>N</i>	%
Age, Median (range)	40 (18-89)	
Age > 45 years	55	39
Male	73	52
Stage		
I-II	77	55
III-IV	63	45
Albumin < 40 g/dL	62	50
Hemoglobin <105 g/L	18	13
ALC < 600	12	9
WBC > 15,000	50	36
B Symptoms	51	36
Bulky Disease	15	11
ECOG PS > 1	16	11
Elevated ESR	53	52
Bone Marrow Involvement	11	8
Splenic Involvement	17	12
IPS		
0-1	45	32
2	36	26
3	33	24
4-7	25	18
Initial Treatment		
ABVD	121	86
MOPP	3	2
Stanford V	5	4
Other/Missing	13	9
Stage by GHSG criteria		
Advanced	44	31
Early favorable	27	19
Early unfavorable	69	49

ALC, absolute lymphocyte count; WBC, white blood cell; ECOG PS, ECOG performance status; ESR, erythrocyte sedimentation rate; IPS, International Prognostic Score; ABVD, adriamycin, bleomycin, vinblastine, dacarbazine; MOPP, mechlorethamine, oncovin, procarbazine, prednisone; GHSG, German Hodgkin Study Group

Table 2

Elevated cytokines in cHL

Cytokine	cHL compared to Controls			
	Controls (n=50)	cHL (n=140)	Rank Sum p-value	% Above 95 th percentile ^b
EGF	84.7 ^a (10.0-902.3)	142.2 (10.0-1002.8)	2.48E-04	16.4
FGF	11 (11.0-285.7)	11 (11.0-201.7)	7.39E-03	10.7
GCSF	133 (10.0-1471.8)	171.9 (10-1383.2)	1.50E-02	12.1
HGF	462.0 (25.0-5443.7)	717.0 (50.0-3075.8)	6.34E-07	18.6
IL-6	1.5 (1.5-283.9)	13.1 (1.5-1429.6)	8.04E-06	19.3
IL-8	21.6 (1.5-334.8)	36.5 (1.5-13333.0)	3.02E-04	28.6
IL-12p40	215.5 (92.5-943.5)	262.4 (96.2-2386.4)	8.91E-05	15.7
IL-2R	400.5 (23.5-3763.0)	1188.5 (183.2-26670.7)	2.67E-13	52.9
IP-10	69.6 (20.4-148.9)	67.8 (13.7-2322.7)	6.36E-01	20.0
MIG	50.3 (15.2-207.9)	98.9 (3.0-2555.0)	1.29E-07	40.7
TNFα	2.5 (2.5-138.2)	2.5 (2.5-332.9)	1.24E-01	10.7
VEGF	5.4 (2.5-29.9)	12.0 (2.5-56.3)	2.04E-04	20.7

^a Values listed as median (range).^b 95th percentile obtained from reference population

Table 3

Elevated cytokines associated with event-free survival in cHL

Cytokine	% Above 95 th percentile ^b	HR ^a	95% CI	P Value
HGF	18.6	3.57	1.60-7.98	0.0019
IL-6	19.3	6.15	2.80-13.53	6.2×10 ⁻⁶
IL-2R	52.9	4.08	1.53-10.89	0.0049
IP-10	20.0	3.23	1.45-7.20	0.0041
MIG	40.7	2.47	1.11-5.50	0.027
sCD30	57.1	3.99	1.36-11.75	0.012

^aHR is elevated vs. nonelevated cytokine;^b95th percentile obtained from reference population