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Elevated serum monoclonal and polyclonal free light chains and Interferon inducible protein-10 (IP-10) predicts inferior prognosis in untreated diffuse large B-cell lymphoma

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Abstract

The detection of serum free light (FLC) is useful in the diagnosis of several hematological diseases. The role and biological relevance of monoclonal or polyclonal FLC elevations in predicting long-term outcome in diffuse large B-cell lymphoma (DLBCL) is unknown. We determined the relationship of the type of FLC elevations to outcome, tumor genotype, and pattern of serum cytokine elevations in 276 patients with untreated DLBCL. Elevated FLC was an adverse prognostic factor through 6 years of follow-up (monoclonal, EFS HR = 3.56, 95% CI: 1.88-6.76, $p < 0.0001$; polyclonal, EFS HR = 2.56, 95% CI: 1.50-4.38, $p = 0.0006$). 73% of DLBCL tumors with monoclonal FLC elevations were activated B-cell type (ABC) vs. 33% from patients with normal FLC. Only ABC-DLBCL lines secreted kappa FLC in vitro and this secretion could be inhibited by the NF- κ B inhibitor bortezomib. Patients with monoclonal FLC had significantly (all $p < 0.001$) elevated serum levels of IL-12, sIL-2R α , IL-1R, and IP-10. Patients with polyclonal elevations of FLC had higher levels of IL-6 ($p = 0.033$), IL-8 ($p = 0.025$), sIL2R α ($p = 0.011$), and IL-1R1 ($p = 0.041$). The combination of elevated FLC and a CXC superfamily chemokine IP-10 predicted a particularly inferior outcome characterized by late relapse. These elevated abnormal FLC and cytokines are potentially useful biomarkers for prognosis and selecting agents for untreated DLBCL.

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Authorship: Contribution: TEW interpreted the data, wrote the paper and provided clinical samples. MM performed all the statistical analysis and interpreted the data. MS performed lab based experiments. CL assisted in statistical analysis. WM performed IHC and provided clinical samples. BL provided clinical specimens. JAK performed FLC assay. MG designed and supervised the research, analyzed and interpreted data, finalized the figures and wrote the paper.

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Keywords

Free light chains; diffuse large B-cell lymphoma; cytokine; IP-10

Introduction

Normal human serum contains a small amount of free kappa and lambda immunoglobulin light chains that are not attached to the immunoglobulin heavy chain. These free light chains can be quantitated by the free light chain (FLC) assay.^{1,2} FLC elevations can be polyclonal (increase in one or both light chains with a normal $\kappa:\lambda$ ratio) or monoclonal (elevation of one FLC that results in an abnormal ratio). The detection of monoclonal FLC is very useful in the diagnosis and management of patients with multiple myeloma and amyloidosis.³ The polyclonal elevation of FLC is also important and is associated with all cause mortality and impaired renal function.^{4,5} We have applied the serum FLC assay to hematologic lymphoid malignancies other than myeloma and found monoclonal FLC in 13% of patients with a variety of lymphoma types.⁶ The incidence of monoclonal FLC was highest in mantle cell (36%) and small lymphocytic (24%) lymphomas; it was found in 8% of those patients with diffuse large B-cell lymphoma (DLBCL).⁶ In a further study of 295 patients with new, untreated DLBCL from two independent cohorts, 32% of patients had an elevated serum FLC (polyclonal or monoclonal) and 14% were monoclonal. Either type of FLC elevation resulted in an inferior prognosis for DLBCL.⁷

In DLBCL, tumors can now be subtyped into activated B cell (ABC), germinal B cell (GBC) or primary mediastinal B cell type by gene expression profiling (GEP).⁸⁻¹⁰ These molecular subtypes have been shown to be prognostic in some datasets and have been very helpful in unraveling the molecular pathogenesis of DLBCL.¹¹ DLBCL genotyping has potential treatment implications and studies are ongoing to learn if certain new treatment regimens are more effective within a specific DLBCL genotypic classification. Immunohistochemistry (IHC) algorithms have been published that demonstrated an association between IHC and gene expression classifications.^{11,12}

In this report, we studied the relationship of the type of FLC elevation to DLBCL genotype, survival parameters, and pretreatment serum cytokine elevation. In addition, we explored the *in vitro* secretion of FLC and cytokines by DLBCL cells and whether pathway-specific drugs could inhibit FLC secretion. It was our hypothesis that ABC-type DLBCL tumors would be more likely to secrete monoclonal FLC because of their known increased content of cells expressing IRF-4 (MUM1) a marker of plasma cells.¹³

Patients and Methods

Patient

Newly diagnosed patients with DLBCL were prospectively enrolled in the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource (MER)^{7,14} or the NCCTG clinical trial N0489.¹⁵ These studies were approved at the Institutional Review Board and all patients signed informed consent to have their samples used for research. This report

contains updated FLC results from the subset of patients receiving immunochemotherapy the 295 patients from the cohort previously published.⁷

DLBCL Cell lines

Human DLBCL cell lines were used to study *in vitro* secretion of FLC by molecular subtype. The GCB lines SUDHL6 (DHL6), OCI-Ly7 (Ly7), OCI-Ly1 (Ly1), and ABC lines OCI-Ly3 (Ly3), SUDHL2 (DHL2), HBL1, and OCI-Ly10 (Ly10) were a gift from the L. Staudt lab (NCI, Bethesda) and maintained in IMDM with 20% human serum (except DHL6, which was grown in RPMI+10% FBS). CD19 cells were purified from peripheral blood mononuclear cells and used as a normal B cell control for FLC analysis. CD19 cells were further cultured in RPMI with 10% fetal bovine serum for FLC analysis.

SUDHL2 and HBL1 cell lines were treated with bortezomib (Sigma-Aldrich) or TG1013458 (Sanofi Aventis) for 24 hours and FLC analysis was performed on the supernatants.

Free light chain assay

Serum FLC was quantitated from enrollment research serum using the FREELITE assay (The Binding Site, Ltd., Birmingham, UK). The FLC assays were performed by the Mayo Clinic Clinical Immunology Lab using kits provided courtesy of The Binding Site. Abnormal κ/λ FLC ratio was *a priori* defined as a κ/λ FLC ratio outside of (0.26, 1.65) and elevated FLC as a κ concentration higher than 1.94 mg/dL or λ concentration higher than 2.63 mg/dL based on the published normal ranges for Mayo Medical Laboratories.¹⁶ A monoclonal elevation of FLC was defined as an elevated FLC with the corresponding FLC ratio outside the reference range (0.26-1.65). Polyclonal elevation of FLC was defined as an elevation of either or both κ or λ FLC outside the laboratory normal range, but with a normal ratio. Abnormal ratios without elevation of either FLC were considered normal based on our previous studies indicating these values were not prognostic in DLBCL.⁷

Immunohistochemistry (IHC)

IHC staining was performed on paraffin tissue from research tissue microarrays (TMAs). All cases were reviewed centrally by the study hematopathologists. DLBCL cases were classified into GCB or non-GCB molecular type based on the Hans, Tally, and Choi algorithms applied to paraffin-embedded tumor samples.¹¹

30-plex ELISA from patient serum

Multiplex ELISA (30-plex) was performed as previously described on available pretreatment patient serum.¹⁷ The cytokine values have been previously published¹⁸ but the data on the relationship of cytokine elevations with monoclonal FLC secretion has not been previously reported.

Cytokine Secretion by DLBCL Cell Lines

Supernatants from various DLBCL cell line cultures were analyzed for secretion using the human sIL-2R α , IL-12, IL-1R1 immunoassay kit (R&D Systems). The specimens were run neat and the end point read at 450 nm using a SpectraMax190 microplate reader (Molecular Devices).

Statistical analyses

Event free survival (EFS) was defined as the time from diagnosis until progression, relapse, re-treatment, or death due to any cause. Analyses of EFS were limited to patients receiving immunochemotherapy. Associations between FLC and EFS were assessed using Cox proportional hazards models and Kaplan-Meier curves. Associations between cytokines and FLC patient groups were assessed using linear models for log₂ scaled cytokine levels and adjusted for study (MER vs. ER-CHOP).

Results

Clinical outcome in monoclonal vs. polyclonal FLC elevation in DLBCL

The patient characteristics and description of FLC abnormalities for the 295 DLBCL cases have been previously reported.⁷ Patients with primary central nervous system lymphoma, post-transplant lymphoproliferative disorders and cutaneous DLBCL were excluded for this study, leaving 276 patients for this analysis. Ninety-four (34%) patients had an elevation of FLC - 68 (25%) polyclonal and 26 (9%) monoclonal. Updated survival curves in patients receiving immunochemotherapy (n=256) show a continued association between both monoclonal (EFS HR = 3.56, 95% CI: 1.88-6.76, p<0.0001) and polyclonal (EFS HR = 2.56, 95% CI: 1.50-4.38, p=0.0006) FLC abnormalities and inferior outcome in newly diagnosed DLBCL (Figure 1). Within the abnormal FLC category patients with monoclonal FLC elevation fared slightly worse (HR=1.38, 95% CI: 0.69-2.74) than patients with polyclonal FLC elevation (p=0.36). Of note, this extended analysis demonstrates that in patients with FLC abnormalities events continue to occur out to 6 years of follow-up whereas a plateau occurs in those DLBCL patients with normal FLC. This in part reflects the effects of co-morbidities that occurred in patients with polyclonal FLC elevations.

Correlation of monoclonal and polyclonal FLC with DLBCL molecular subtypes

Tissue for IHC staining to determine molecular classification was available on 121 of the 276 cases (44%); 75 (63%) were GCB and 45 (38%) were non-GCB per Hans algorithm. Of these 121 cases 83 had normal FLC; 11 had monoclonal FLC; and 27 had polyclonal FLC. By the Hans, Choi, and Tally algorithms, 73% (8/11), 70% (7/10), and 90% (9/10) of these cases were ABC-type DLBCL, respectively (Table 1). The percentage of ABC patients by these same algorithms in the corresponding DLBCL patients with normal FLC secretion was 33% (p=0.0096), 38% (p=0.053), and 46% (p=0.0091), respectively. Patients with polyclonal FLC secretion had similar IHC-determined genotypes as those with normal FLC (both p >0.57). Abnormal FLC secretion was associated with inferior outcomes in both GCB and non-GCB groups.

Secretion of FLC by DLBCL Cell Lines

Supernatants from cell cultures of normal CD19 B cells and GCB and ABC-type DLBCL cell lines were analyzed for FLC secretion. Normal CD19 B cells did not secrete either κ or λ light chain into the culture media (Figure 2A). The GCB-type DLBCL cell lines (DHL6, Ly7, and Ly1) secreted small amounts of FLC similar to normal human serum. In contrast,

the three ABC-type DLBCL cell lines tested (DHL2, HBL1, and Ly10) secreted high amounts of κ FLC with an abnormal FLC ratio (Figure 2B, 2C).

Effect of NF- κ B and JAK2 Pathway Inhibition on FLC secretion

The NF- κ B and STAT3 pathways are activated in ABC-type DLBCL lymphoma.¹⁹⁻²³ It has also been shown that the ABC DLBCL genotype is sensitive to NF- κ B or JAK/STAT inhibitors.^{19,22} We investigated whether *in vitro* treatment of the DLBCL cell lines with bortezomib (an NF- κ B inhibitor), or TG1013458 (JAK2 inhibitor) could decrease FLC secretion in ABC-type DLBCL cell lines. Treatment with sublethal doses of bortezomib, but not TG1013458, decreased κ FLC secretion in the DHL2 and HBL1 cell lines in a dose dependent manner (Figure 3A,B).

Correlation of abnormal (monoclonal and polyclonal) FLC with pretreatment serum cytokine level

We have previously demonstrated that patients with DLBCL have an abnormal cytokine profile that can be prognostic for relapse and survival.^{17,18} Cytokine levels from pretreatment serum were compared to the FLC data available from the same time point (Table 2). Patients with monoclonal FLC had significantly elevated concentrations of IL-12 (p=0.000087), sIL-2R (p=0.00011), IL-1R1 (p=0.00024), and IP-10 (p=0.00010). Patients with polyclonal elevations of FLC had higher levels of IL-6 (p=0.033), IL-8 (p=0.025), sIL2R α (p=0.011), and IL-1R1 (p=0.041).

In-vitro secretion of sIL-2R α , IL-12, IL-1R1 and IP-10 by DLBCL cell lines

Supernatants from the GCB DLBCL cell lines DHL6, Ly1 and Ly7 and the ABC cell lines DHL2, Ly3, Ly10 and HBL1 were collected and assayed for key cytokines that were significantly correlated with monoclonal FLC (Table 2); namely, IL-12, sIL-2R α , IL-1R1 and IP-10 by individual ELISA (Figure 4). All the 3 GCB cell lines secreted detectable sIL-2R α (range;100-445 pg/ml), whereas only one of the ABC cell lines (Ly3) secreted sIL-2R α (284, pg/ml) as compared to Human serum alone. With respect to IL-12, only 1 GCB cell line (Ly7) and 2 ABC cell lines (DHL-2 and Ly3) secreted detectable amounts. Secretion of IL-1R1 was very low in all cell lines (<20 μ g/ml) with the exception in Ly3 cells (45 pg/ml). IP-10 was not secreted by none of the GCB lines (data not shown), however 2/3 ABC lines (DHL2, Ly3) secreted significant amount of IP-10 (Figure 4). In summary, sIL2R α is secreted *in-vitro* primarily by GCB DLBCL cell lines; IP-10 by ABC-type, IL-12 by both types, and IL-1R1 by neither type.

In-vivo correlation of IP-10 and monoclonal FLC for EFS

We have previously shown that high IP-10 is associated with inferior EFS in DLBCL and with elevated FLC in general.¹⁸ In the current study we evaluated the relationship of IP-10 with type of FLC secretion. We found that the majority (81%) of patients with monoclonal FLC also had a high IP-10 (greater than the median), compared to 51% and 45% of the patients with polyclonal or normal FLC, respectively. Patients with elevations of both IP-10 and FLC (polyclonal or monoclonal) had very poor EFS (HR=5.47, 95% CI: 3.07-9.73, p=7.36 \times 10⁻⁹) compared to patients with both parameters low or normal (Figure 5). Patients

with either high IP-10 or elevated FLC but not both had an intermediate outcome (HR=2.21, 95% CI: 1.27-3.83, p=0.0050).

Discussion

The era of targeted therapy has made available many new treatments for relapsed DLBCL. Agents such as epratuzumab,²⁴ bortezomib,²⁵ enzastaurine,²⁶ lenalidomide,^{27,28} and everolimus²⁹ have all demonstrated single-agent activity in relapsed DLBCL. These agents are now being combined with standard R-CHOP. Epratuzumab,¹⁵ bortezomib,^{30,31} and lenalidomide³² have all competed phase I/II trials with encouraging safety and efficacy. The current challenge is to demonstrate whether any of these regimens are superior to RCHOP alone. With the multiple agents to be tested it will be very helpful to have biomarkers that reflect tumor biology to select which patients stand to benefit from the addition of a specific new agent.

In this report we provide long-term follow-up on the prognostic relevance of elevated serum FLC and the type of elevation in untreated DLBCL. This analysis reveals that patients with elevated FLC continue to relapse even after the first two years; whereas, the relapse curve for patients with normal FLC follows the more conventional plateau after the first 2 years.³³ A recent report by Jardin et al.³⁴ provides important confirmation of the clinical utility of serum FLC abnormalities in DLBCL. They studied 407 patients with untreated DLBCL for serum FLC and immunoglobulin heavy chain/light chain pairs (HLC). Consistent with our data, patients with abnormal FLC had an inferior PFS and OS compared to those with normal ratios. The finding that abnormal IgM κ /IgM λ ratios are also prognostic is new and not previously reported for DLBCL. The production of serum FLC in patients with DLBCL could be derived from microenvironment cells based on stimuli from tumor cells or from the tumor cells themselves. In multiple myeloma the monoclonal plasma cells secrete the FLC whereas in Hodgkin lymphoma polyclonal FLC are secreted by the benign cells in the tumor microenvironment.³⁵ Although we found no statistical difference from a prognostic standpoint between polyclonal or monoclonal FLC elevations (they both predicted inferior outcome), there was a trend for monoclonal FLC patients having the worst prognosis and there were other biological differences. The finding of a monoclonal FLC predicted for ABC-type DLBCL by IHC; normal and polyclonal FLC patterns did not inform the genotype. Only ABC-type DLBCL cell lines secreted monoclonal FLC. These results are based on a relatively small dataset and require confirmation in large randomized trials and additional DLBCL cell lines require testing. Our studies determined genotype by the typical IHC algorithms that have shown reasonable correlation with GEP.¹¹ The tumors from patients with monoclonal FLC were for the most part classified as ABC-type by all three algorithms. Jardin et al.³⁴ also classified a subset (n=199) of their cases into GCB and non-GCB by Hans algorithm. Sixteen percent (15/94) of the patients with GCB type DLBCL had an abnormal HLC ratio compared to 23% (24/105) of non-GCB cases (p=0.2). In addition, 53 cases had GEP and 16 of these had an abnormal HLC ratio (5 were GCB and 11 were ABC, p=0.14) and 8 patients had an abnormal FLC ratio (genotype not reported). Thus, there was a trend towards abnormal ratio FLC or HLC being non-GCB type. Future prospective studies should correlate monoclonal FLC and HLC secretion with DLBCL genotype to confirm or deny this relationship. Until these studies are performed, the FLC

and HLC assays will primarily be used as prognostic factors. The fact that the assay is readily available in the clinical laboratory on a daily basis makes it ideal for use as a stratification factor for randomized clinical trials. The trend of monoclonal FLC to be associated with non-GCB-type DLBCL is also potentially useful to initially stratify patients on trial until GEP classification is available.

The serum cytokine profile assayed at the same time point as the FLC also differed by the pattern of FLC abnormality. Patients with monoclonal FLC had significantly elevated concentrations of sIL-2R, IL-12, IL-1R1 and IP-10 cytokines. IL-1R1 and sIL2R elevations were found associated with both polyclonal and monoclonal FLC. Many groups have found sIL2R elevations to be associated with an adverse prognosis in DLBCL.³⁶⁻⁴¹ Serum IL12 is also recognized to be elevated in DLBCL but was not a strong prognostic factor.¹⁸ The high IL-12 levels we found were more likely in patients with monoclonal FLC secretion. In B-cell lymphoma IL-12 has been demonstrated to be detrimental by causing T-cell exhaustion.⁴² IP-10, also known as CXCL10, is a pro-inflammatory cytokine associated with inferior DLBCL prognosis.¹⁸ In this study, IP-10 was associated with monoclonal FLC secretion and when IP-10 and abnormal FLC were combined they predicted an especially poor outcome.

In the future, treatment approaches for DLBCL are likely to use the RCHOP-21 backbone with additional agents being selected based on a specific genotype or signal pathway activation profile. Large randomized trials are ongoing that use genotype in the selection and statistical plan to test the hypothesis that new combinations are superior in the non-GCB genotype. Our cell line data demonstrating the ability of the NF- κ B inhibitor bortezomib to reduce FLC secretion from ABC-type cell lines is in support of the current European trial of RCHOP + bortezomib vs. RCHOP for patients with non-GCB type DLBCL (NCT01324596). The cytokine secretion results in these cases also offer clues as to potential therapeutic interventions. The finding of elevated IL-1R1 in a subset of DLBCL patients with monoclonal FLC is potentially actionable with the IL-1R antagonist anakinra.⁴³ sIL-2R in monoclonal FLC elevations may be amenable to the addition of basiliximab, a monoclonal antibody to the IL2R α -chain (CD25) used primarily in renal transplantation to prevent acute rejection.^{44,45} The addition of these inhibitors or blockers of cytokine action may potentially relieve lymphoma related symptoms (fevers, night sweats, weight loss) and fatigue in addition to improving response to therapy. However, proof of this principle will require large randomized trials with cytokine measurements pre- and post-intervention. In the meantime, abnormal FLC and cytokines/chemokines measurements are important for prognosis and stratification in clinical trials.

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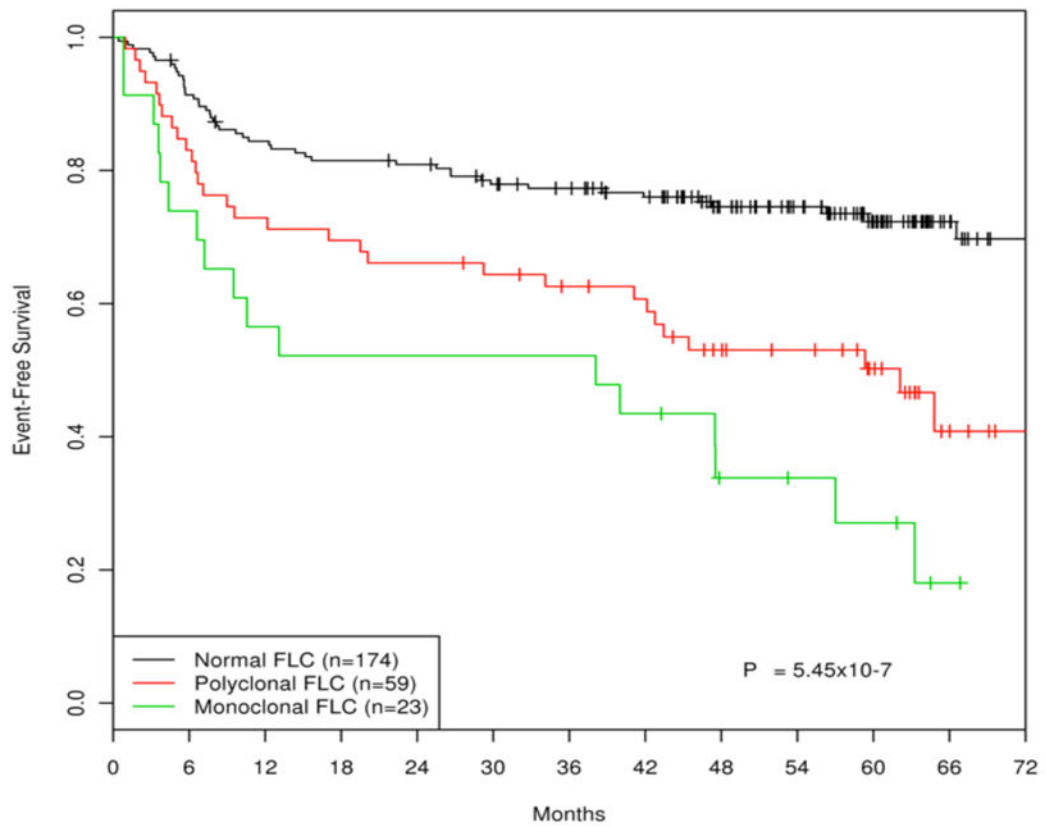


Figure 1. Event-free survival by normal, polyclonal and monoclonal FLC secretion of patients with untreated diffuse large B-cell lymphoma treated with chemoimmunotherapy (n=256).

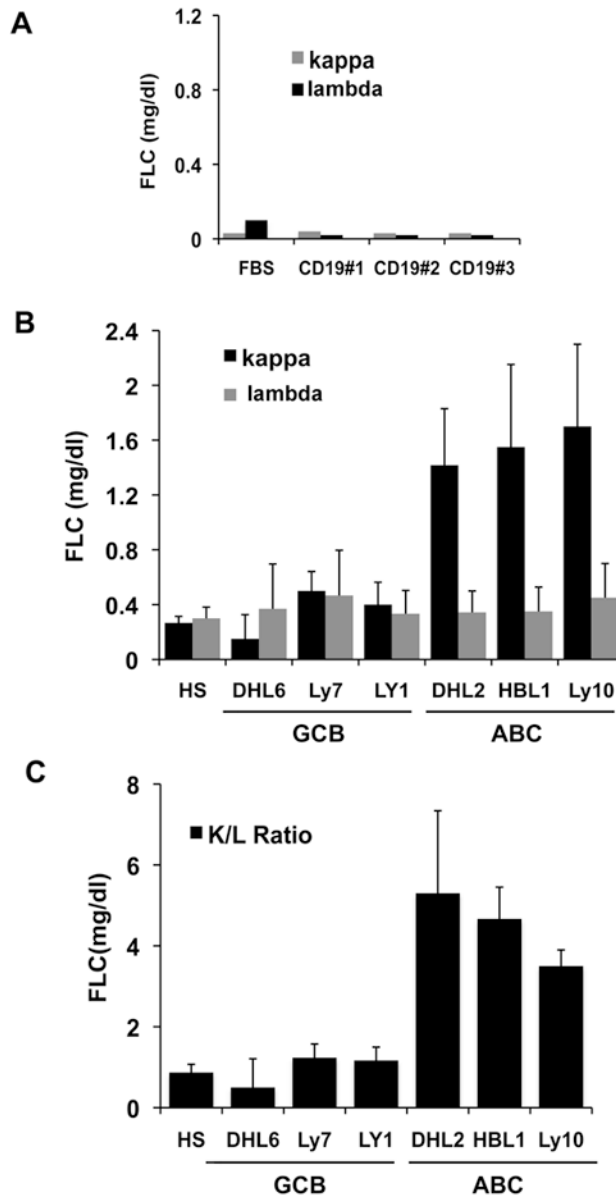


Figure 2. In-vitro secretion of FLC by normal B (CD19+) and DLBCL cells. (A) FLC secretion by CD19+ B-cells (n=3) from the blood of normal controls comparable to fetal bovine serum (FBS) (B) FLC (kappa and lambda) secretion by ABC and GCB DLBCL human cell lines compared to human serum control (HS). (C) FLC (kappa/lambda ratio) secretion in ABC and GCB DLBCL cell lines. Bars represent mean \pm SD from three separate experiments.

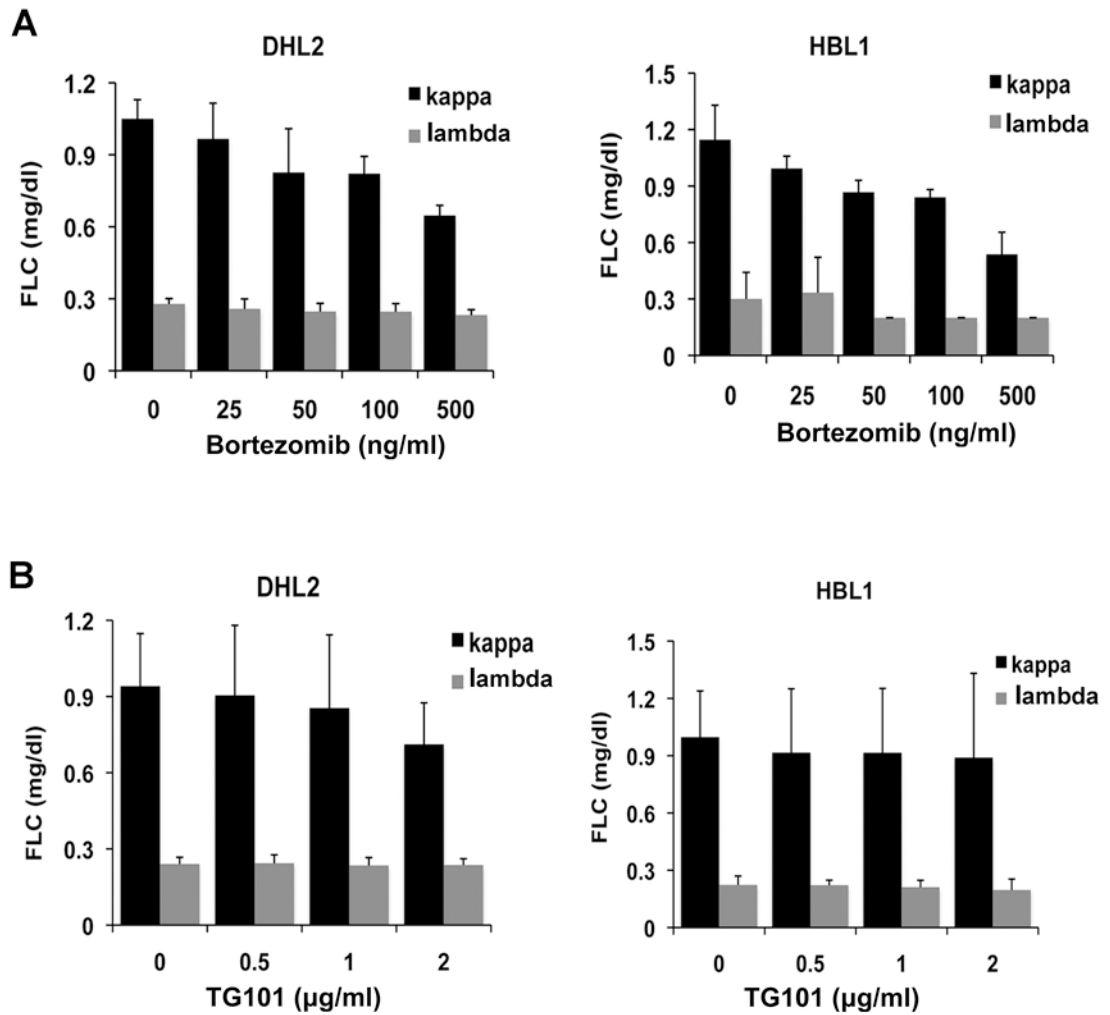
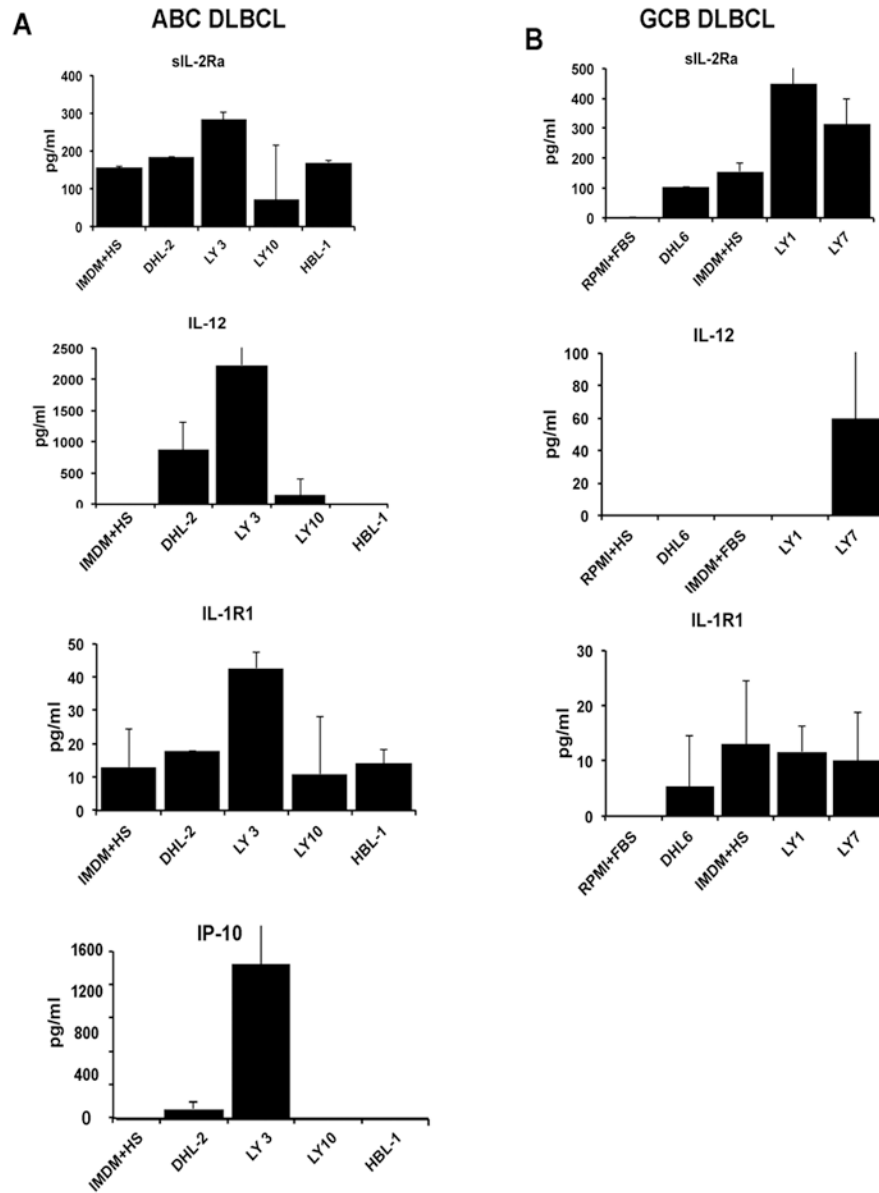


Figure 3.

(A-B): Effect of the NF- κ B inhibitor bortezomib (A) and the JAK2 inhibitor TG101 (B) on monoclonal kappa FLC secretion by the ABC DLBCL cell lines DHL2 and HBL1. Bars represent mean \pm SD from three separate experiments.

**Figure 4.**

In vitro secretion of elevated cytokines (sIL-2R α , IL-12, IL-1R1 and IP-10) correlated with monoclonal FLC in DLBCL supernatants. 3.0×10^6 cells from ABC (**A**) and GCB (**B**) DLBCL cell lines were cultured for 48 hours. Supernatant was then collected and ELISA was performed for sIL-2R α , IL-12, IL-1R1 and IP-10 as described in Material and Method section. Human serum and fetal bovine serum were used as control. Bars represent mean \pm SD from three separate experiments.

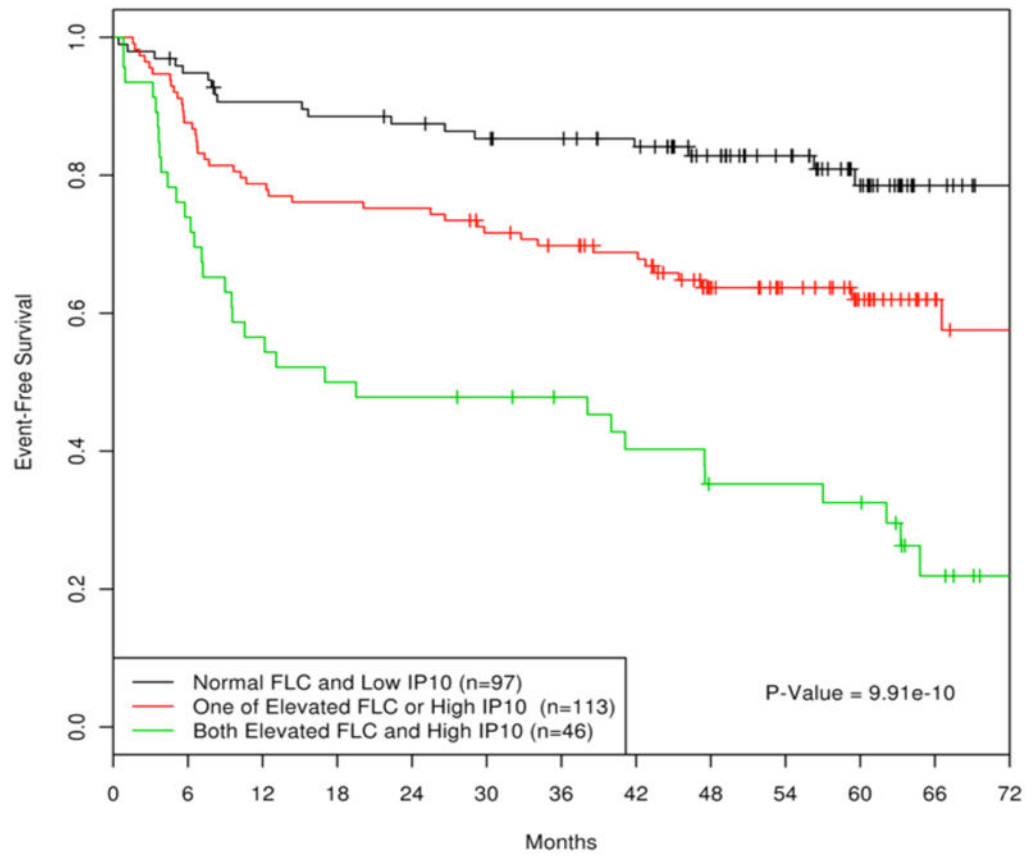


Figure 5. Event-free survival in DLBCL (n=256) by pre-treatment serum FLC and elevated serum IP-10.

Immunohistochemistry markers in 11 patients with diffuse large B-cell lymphoma and monoclonal serum immunoglobulin free light chain secretion.

Table 1

Case	Serum Kappa	Serum Lambda	Serum FLC Ratio	Germinal center			ABC		Either	Algorithm		
				CD10	GCE11	LMO2	MUM1	FOXPI		BCL6	Hans	Choi
NCCTG1	3.38	2.02	1.67	-	-	+	+	-	+	ABC	GCB	ABC
NCCTG2	5.57	0.43	13.1	-	-	-	-	+	-	ABC	ABC	ABC
NCCTG3	3.68	1.61	2.29	+	-	-	+	+	-	GCB	GCB	ABC
MER1	4.87	2.11	2.31	-	ND	-	-	ND	-	ABC	NA	ABC
MER2	4.51	1.57	2.87	-	-	-	-	-	-	ABC	ABC	ABC
MER3	43.6	1.23	35.4	-	-	-	+	+	-	ABC	ABC	ABC
MER4	2.83	1.47	1.93	-	-	-	-	+	+	GCB	ABC	ABC
MER5	5.16	0.36	14.3	-	-	ND	-	ND	-	ABC	ABC	N/A
MER6	8.3	4.85	1.71	-	-	-	+	-	-	ABC	ABC	ABC
MER7	1.26	9.67	0.13	-	-	-	-	ND	-	ABC	ABC	ABC
MER8	45.7	5.4	8.46	+	-	+	-	+	+	GCB	GCB	GCB

Abbreviations: ABC – activated B-cell cell type; FLC, free light chain; NCCTG, North Central Cancer Treatment Group (now ALLIANCE); MER, Molecular Epidemiology Resource

Table 2

Cytokine values (median, min, max) are log₂ transformed and batch-adjusted from pg/ml raw values. P-value is from the Wilcoxon rank-sum test comparing to normal serum immunoglobulin free light chain (FLC). Normal FLC data include 15 patients with non-elevated FLC with abnormal FLC ratio (i.e. ratio-only). Shaded cytokines are significantly correlated with monoclonal FLC.

Cytokine	Normal FLC (N=157)			Polyclonal Elevated FLC (N=57)			Monoclonal Elevated FLC (N=23)			
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	p-value
EGF	6.5	5.4	7.1	6.4	5.4	7.2	6.4	5.6	7.2	0.837
Eotaxin	6.9	6.4	7.4	7.0	6.4	7.5	7.0	6.5	7.4	0.923
FGF-β	4.5	4.0	5.2	4.5	4.0	5.1	4.5	3.8	5.6	0.762
GM-CSF	2.3	2.0	5.2	2.3	1.7	5.6	2.3	1.5	3.8	0.088
G-CSF	5.3	4.3	7.8	6.5	4.4	8.0	6.5	4.5	7.8	0.079
HGF	9.1	8.4	9.7	9.4	8.7	10.2	9.5	8.4	10.3	0.216
IFN-α	6.0	4.7	6.8	5.9	4.6	6.7	6.2	4.7	6.7	0.764
IFN-γ	2.3	2.0	3.6	2.7	2.3	4.4	2.3	2.0	3.8	0.592
IL-2	2.7	2.1	3.8	2.8	1.6	3.9	3.3	2.0	4.3	0.602
IL-4	4.9	2.3	6.2	4.9	2.3	6.4	5.3	2.6	6.4	0.266
IL-5	1.6	1.6	3.4	2.0	1.6	3.8	1.6	1.6	3.6	0.847
IL-6	2.9	1.6	4.7	4.4	1.6	5.9	4.6	1.6	5.5	0.062
IL-7	3.3	3.3	5.4	3.3	3.3	4.7	3.3	3.3	6.3	0.997
IL-8	5.4	4.5	6.2	5.7	5.0	6.7	5.8	5.1	6.6	0.075
IL-10	4.6	3.6	5.6	4.7	2.9	5.7	5.2	4.1	5.8	0.051
IL-13	4.6	1.7	5.4	4.8	1.3	5.4	4.6	1.7	5.3	0.844
IL-15	3.3	3.3	6.3	3.4	3.3	6.5	3.3	3.3	7.0	0.367
IL-17	4.0	3.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0	0.028
IL-12	8.0	7.4	8.6	8.3	7.7	8.7	8.8	8.3	9.4	0.000087
sIL-2Rα	9.9	9.0	11.0	10.6	9.4	12.0	11.5	10.6	12.9	0.00011
IL-1R1	9.4	8.6	10.3	9.8	9.1	10.5	10.2	9.5	11.4	0.00024
IL-1β	3.9	2.9	4.6	3.9	2.9	3.9	3.9	3.9	6.2	0.148
IP10	6.3	5.2	7.3	6.7	5.5	7.6	7.4	6.3	8.9	0.00010
MCP-1	9.5	9.1	10.0	9.8	9.2	10.2	9.7	9.5	10.2	0.067

Cytokine	Normal FLC (N=157)			Polyclonal Elevated FLC (N=57)			Monoclonal Elevated FLC (N=23)			
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	p-value
MIG	7.4	6.0	8.9	8.2	6.8	9.1	8.6	6.6	10.1	0.097
MIP-1 α	5.8	4.7	6.8	6.0	5.2	7.0	6.3	5.6	7.0	0.023
MIP-1 β	6.6	6.0	7.2	6.8	6.2	7.4	6.8	6.2	7.9	0.183
RANTES	12.8	2.9	13.0	12.7	2.9	12.9	12.8	12.3	13.0	0.920
TNF- α	2.3	1.7	2.4	2.3	1.3	2.3	2.3	2.0	2.3	0.723
VEGF	2.3	1.3	3.3	2.6	1.3	3.9	2.6	1.8	3.9	0.124