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A Novel Prognostic Model for Osteosarcoma Utilizing Circulating CXCL10 and FLT3LG

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Abstract

Background—Osteosarcoma is the most common malignant pediatric bone tumor. Identification of novel biomarkers for early prognostication will facilitate risk-based stratification and therapy. In this study, we investigated the significance of circulating cytokines/chemokines to predict prognosis at initial diagnosis.

Experimental design—We employed Luminex assays to measure cytokine/chemokine concentrations from blood samples in a discovery cohort of osteosarcoma patients from Texas Children's Hospital (n= 37) and an independent validation cohort obtained from the Children's Oncology Group (n= 233). Following validation of biomarkers, we constructed a multivariable model to stratify patients into risk groups.

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Results—The circulating concentrations of CXCL10, FLT3LG, IFNG, and CCL4 were significantly associated with overall survival in both cohorts. Of these candidates, CXCL10 and FLT3LG were independent from the existing prognostic factor, metastasis at diagnosis, and CCL4 further discriminated cancer-versus-control. By combining CXCL10, FLT3LG and metastatic status at diagnosis, we developed a multivariate model that significantly stratified the patients into four distinct risk groups ($p = 1.6e-8$). Survival analysis showed that 5-year overall survival rates for the “Low”, “Intermediate”, “High” and “Very-high” risk groups were 77%, 54%, 47%, and 10% respectively, while 5-event-free survival rates were 64%, 47%, 27%, and 0% respectively. Neither CXCL10 nor FLT3LG tumor expression were significantly associated with survival.

Conclusions—High circulating levels of CXCL10 and FLT3LG predicted worse survival in osteosarcoma. Since both CXCL10 and FL3LG axes are potentially targetable, further study may lead to novel risk-based stratification and therapy in osteosarcoma.

Keywords

osteosarcoma; metastasis; CXCL10; FLT3LG; circulating biomarkers; Luminex; pediatric cancer and prognosis

Introduction

Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents¹. Currently, 10-year overall survival is 60-70% for all patients² and only about 20% for patients with metastatic disease³, a known prognostic factor at the time of diagnosis. The other clinical prognostic factor is response to neoadjuvant chemotherapy and is measured at the time of definitive surgery. Survival of metastatic patients has not significantly improved in recent decades, mainly because therapeutic options for metastasis are very limited. Therefore, all OS patients receive the same chemotherapy at diagnosis despite their clinical risks. Additionally, altering post-operative chemotherapy based on response to neoadjuvant chemotherapy has not improved outcomes, highlighting the importance of tailoring treatment at diagnosis rather than after chemotherapy resistance has developed⁴. We believe a promising strategy is to discover biomarkers for high-risk OS patients, where the biomarker-associated pathways can be potentially targeted.

Although many previous studies have focused in tumors, blood-based biomarkers have become increasingly important in cancer research⁵. Circulating biomarkers can be obtained through noninvasive procedures and contain molecular information not only from the tumor, but also the host immune system and tumor microenvironment. In OS, levels of serum alkaline phosphatase⁶ and lactate dehydrogenase⁷ have been proposed as prognostic biomarkers. More recently, circulating miRNA levels, i.e. miR-195-5p, miR-320a, have been proposed for early detection and tumor burden monitoring⁸. However, no biomarkers are currently used to guide clinical decisions and therapies in clinic.

Our group previously identified CXC chemokines that were significantly elevated in OS patient peripheral blood and tumors⁹. We further identified that the “cell adhesion/chemokines and adhesion” pathway was commonly associated with metastatic OS¹⁰. Therefore, we reasoned that cytokines/chemokines might also serve as biomarkers for poor

prognostic OS. Recent studies have demonstrated the roles of these proteins in tumorigenesis and cancer progression¹¹. Specifically, OS research has revealed complex roles of chemokine ligand/receptor axes relating to metastasis and prognosis¹². In this study, we showed that CXCL10, FLT3LG, IFNG, and CCL4 have prognostic significance in OS patients. A combined model with CXCL10, FLT3LG and metastatic status significantly improved prognostic significance compared to metastasis alone.

Materials and Methods

Patients and samples

Plasma samples for the discovery cohort were collected at initial diagnosis from 37 OS patients of Texas Children's Hospital, who consented to participate under an institutional review board-approved protocol. The patients were 5 to 18 years old with a median follow-up time of 25 months. Samples were collected in ethylenediaminetetraacetic acid-containing tubes at room temperature and immediately centrifuged at 1,000 rpm for 10 minutes. Plasma supernatant was stored at -80°C until use. The Children's Oncology Group provided 233 validation OS serum samples collected at diagnosis under three collection/treatment protocols (P9851, P9754 and AOST0121) from multiple institutions. Patients were 2 to 31 years old with a median follow-up of 49 months. Chi-squared tests were used to compare discovery and validation set clinical parameters (Table 1). Twelve serum samples from 18 year-old healthy donors (Equitech-Bio), and five plasma samples from noncancerous pediatric patients (i.e. well-child checkup, flu, constipation, gastroenteritis, and febrile seizure) collected from Texas Children's Hospital were used as normal controls.

Cytokine and chemokine assays

This study employed the Luminex Suspension Bead Array (Luminex Corp., Austin, TX) platform, enabling simultaneous analysis of many proteins per sample, with equal or higher sensitivity to traditional ELISA¹³. Human 39-Plex Cytokine panel (Millipore, Billerica, MA) was used to detect the following cytokines/chemokines: GM-CSF, G-CSF, IFNG, IL-1a, IL-1ra, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-12p40, IL-13, IL-15, IL-17, MCP-1, MCP-3, MDC, TNFa, TNFb, TGFa, Eotaxin, IFNa2, IP-10/CXCL10, MIP-1a, MIP-1b/CCL4, EGF, FGF-2, Flt-3 Ligand/FLT3LG, Fractalkine, GRO, VEGF, sCD40L, and sIL-2Ra. The assay was performed according to manufacturer's protocol. Plates were analyzed using the Bio-Plex 200 system and Bio-Plex manager software 4.1 (Bio-Rad, Hercules, CA). Fifty beads per analyte were collected and fluorescence intensity was recorded. Protein concentrations were calculated based on standard curve data using 5-parameter logistic fitting method.

Immunohistochemistry

Tumor expression of CXCL10 and FLT3LG were measured by immunohistochemistry in a tissue microarray containing 57 osteosarcoma tumor samples with anti-CXCL 10 and anti-FLT3LG antibodies (Abcam, Cambridge, MA). Staining/scoring methods were performed similarly as previously described⁹. The staining score cutoff that best separated patients into two equal groups defined "Low" and "High" expression for each candidate marker. The data was then independently analyzed with respect to overall survival.

Data analysis

Cytokine/chemokine concentrations were Arcsinh-transformed with out-of-range-low values set to 0, and out-of-range-high values set to maximum detected value. In the discovery cohort, an optimal cutoff for each protein was chosen using the Gini-index criterion from the *rpart* package in R¹⁴ for Kaplan-Meier analysis with respect to overall survival. Proteins that stratified patients into “High-risk” and “Low-risk” with the Benjamini-Hochberg corrected log-rank $p < 0.1$ were selected for validation. For validation, the pre-determined cutoff concentrations were used for Kaplan-Meier analysis with respect to overall and event-free survival (EFS) using the one-tailed log-rank statistic. Events were defined as either death or relapse. The validated biomarkers were further analyzed with pairwise Pearson correlations.

A multivariate model was then constructed using the metastasis-only model as baseline and stepwise Bayesian information criterion (BIC) with backward selection to select candidates that improved prognostic prediction. Selected markers were confirmed as independent from initial metastasis by the nested likelihood-ratio test. A final model was constructed by assigning patients risk-scores of 0, 1, 2, or 3 based on number of risk factors present (CXCL10 > cutoff, FLT3LG > cutoff, and/or metastasis at diagnosis). The four risk-groups are defined as “Low”, “Intermediate”, “High”, and “Very-high” risk, corresponding to risk-scores of 0, 1, 2, or 3, respectively. Model significance was determined using the one-tailed log-rank statistic, whereas significance of individual Hazard Ratios (HR) was determined using the Wald statistic. The association of each biomarker with metastasis at diagnosis, response to chemotherapy and cancer-versus-control, were evaluated using Welch's t-tests. Unless specified, $p < 0.05$ was considered significant.

Results

We investigated circulating levels of 39 cytokines/chemokines in discovery and validation cohorts with no significantly different clinical characteristics (Table 1). In the validation cohort, patient survival significantly correlated with the collection/treatment protocol ($p < 0.05$), reflecting the fact that one protocol (AOST0121) contained only metastatic patients, and another (P9754) contained only non-metastatic patients. However, the metastatic and non-metastatic patients from the third protocol (P9851) had similar survival to those in AOST0121 and P9754, respectively, and the protocols did not significantly correlate with overall survival after correcting for metastatic status (Supplementary Figure 1). Furthermore, time of sample collection was not significantly associated with survival.

CXCL10, FLT3LG, IFNG, and CCL4 significantly correlate with survival

In the discovery cohort, 26 proteins were associated with overall survival after multiple-testing correction (Table 2). Of note, expressions of all but one (MDC/CCL22) of the proteins had HR > 1, linking high expression with a poorer outcome.

Using the same concentration cutoffs, these candidate biomarkers were examined in the validation cohort. Results showed that higher levels of CXC motif chemokine 10 (CXCL10, $p = 7.0\text{e-}4$), Fms-related tyrosine kinase 3 ligand (FLT3LG, $p = 4.6\text{e-}3$), Interferon gamma

(IFNG, $p = 0.013$), and Chemokine ligand 4 (CCL4, $p = 0.037$) significantly correlated with poorer overall survival (Table 2). CXCL10, IFNG, and FLT3LG also significantly correlated with EFS (Figure 1). Five-year overall survival and EFS rates of “High” and “Low” biomarker expression groups, and for patients stratified by metastasis, are listed in Table 3. The concentration cutoffs defining the “High” and “Low” expression groups were 328 pg/ml (70th percentile; range = 43–3044) for CXCL10, 16 pg/ml (63rd percentile; range = 0–17,939) for IFNG, 17 pg/ml (77th percentile; range = 0–1780) for FLT3LG, and 50 pg/ml (38th percentile; range = 8–2901) for CCL4. No biomarker significantly differed between OS and control samples other than CCL4 (Fisher's Exact test, OR = 25.6, $p = 5e-6$, Supplementary Figure 2). All biomarkers remained significantly correlated to overall survival when controlling for protocol and time of collection.

In pairwise correlation analysis, FLT3LG, IFNG and CCL4 were highly correlated with each other ($R = 0.5-0.61$), indicating that there may be prognostic redundancy, while CXCL10 showed weak correlation with all others ($R < 0.3$) (Supplementary Figure 3). Upon examination of correlations with known prognostic factors, CXCL10 alone was significantly correlated to metastasis at diagnosis ($p < 0.05$), whereas none of the four biomarkers correlated with histological response to chemotherapy (Supplementary Table 1).

Multivariable model combining CXCL10, FLT3LG, and metastatic status significantly stratifies OS patient survival

In the validation cohort, 5-year overall survival for non-metastatic and metastatic patients at diagnosis was 67% and 44%, and 5-year EFS was 57% and 30% respectively, which is similar to previous studies^{15, 16} (Figure 3, Table 3). We sought to develop a novel stratification model by selecting biomarkers that added prognostic value to metastasis. In both overall and EFS, FLT3LG and CXCL10 increased prognostic value of metastatic status alone based on BIC and validated with the nested likelihood-ratio test (Chi-squared $p < 0.05$, Supplementary Table 2). However, CCL4 and IFNG did not, verifying the expectation from the correlation analysis. We thus combined CXCL10 and FLT3LG into a two-biomarker model, which significantly stratified OS patients with high expression of neither, one or both of CXCL10 and FLT3LG ($p < 0.05$, Figure 2A). Furthermore, this model significantly stratified both metastatic and non-metastatic patients separately by survival ($p < 0.05$, Figure 2B and 2C).

Consequently, we created a multivariate model by adding metastatic status with the two biomarker model, which results in a four risk-group stratification, namely, “Low”, “Intermediate”, “High” and “Very-high” risks. 5-year overall survival rates for these risk-groups were 77%, 54%, 47%, and 10%, respectively, whereas 5-year EFS rates were 64%, 47%, 27%, and 0%, respectively, implicating a final model with greater prognostic significance than metastatic status alone ($p = 1.6e-8$, Figure 3, Table 3).

CXCL10 and FLT3LG are expressed in the majority of OS tissue samples

Using a tissue microarray, we found that 52% and 86% of OS tissues had at least moderate intensity scores for FLT3LG and CXCL10 respectively. Similarly, in 54% and 89% of tissues, at least 50% of tumor cells showed staining for FLT3LG and CXCL10 respectively

(See Figure 4, and Supplementary Table 3). Unlike the blood result, tumor intensity and proportion of neither FLT3LG nor CXCL10 significantly correlated with overall survival (See Supplementary Figure 4).

Discussion

Precision medicine through combining molecular biomarkers and targeted therapies has become increasingly important in modern cancer treatment. Ideally, biomarkers can also act as potential targets, e.g. EGFR mutation and mTOR¹⁷⁻¹⁹. However, because of tumor heterogeneity and genome instability, identification and validation of clinically relevant tumor biomarkers in OS has been very difficult^{1, 20, 21}. Thus, no biomarkers beyond radiographically visible metastasis at diagnosis have yet been translated to the clinic to stratify patients and guide therapeutic options¹⁵. In this study, we demonstrated that high levels of circulating CXCL10 and FLT3LG were significantly associated with poor overall survival and EFS independently of metastasis. We further combined CXCL10, FLT3LG and metastatic status into a prognostic model that significantly stratified patients into four risk-groups. The model defined survival risks significantly better than when using metastasis alone for prognosis, and may serve as a risk-stratification scheme for future clinical trials where high-risk patients can be treated with potential targeted therapies to improve their outcomes.

Our result also showed that both CXCL10 and FLT3LG were expressed in a majority of OS tissues. However, their tumor expressions did not correlate with overall survival. This may be due to the semi-quantitative nature of immunohistochemistry and/or the limited number of samples used in the tissue microarray. Despite these limitations, we observed that higher FLT3LG expression exhibited a trend towards poorer overall survival, which is similar to the circulating FLT3G result. Therefore, larger and more quantitative studies of these two proteins in OS may be warranted.

CXCL10 is secreted in response to IFNG under proinflammatory conditions²². Circulating IFNG levels significantly correlated with survival in this study, but was excluded from the multivariate model, suggesting its prognostic information was explained by CXCL10 and/or FLT3LG levels. Prognostic value of CXCL10 has been demonstrated in other cancers^{23, 24}. It can also serve as a paracrine factor promoting metastasis to target organs, such as lungs, liver and lymph nodes^{25, 26}.

The cognate receptor of CXCL10 is CXCR3, which has multiple functions in cancer²³. Specifically, CXCL10 has higher binding affinities for the CXCR3-A isoform to promote tumor proliferation²⁷. Preliminary data suggests that CXCR3 was expressed in a vast majority of OS tumors (data not shown). CXCR3 antagonist AMG487²⁸ can inhibit metastases in OS murine models²⁹, and targeting CXCR3 inhibits metastasis without adversely affecting anti-tumoral host response in breast cancer³⁰. However, high NK cell infiltration in solid tumors has been reported as a good prognostic factor, and recruitment of CXCR3-positive NK cells to the tumor site promotes survival in mouse models of lymphoma and melanoma^{31, 32}. Therefore, further studies need to be conducted to better understand the therapeutic effect of targeting CXCR3 in OS.

Conversely, FLT3LG is a growth factor that binds to FLT3 (CD135), a tyrosine kinase receptor³³ which has been implicated in cancers and specifically in leukemia³⁴. Increased circulating FLT3LG levels have been observed in subjects with impaired FLT3 receptor signaling³⁵, or following treatment with FLT3-blocking compounds, such as sunitinib³⁶. To our knowledge, impaired FLT3 signaling in OS has not been reported, and patients in this study were not treated with FLT3-blocking agents. Although we demonstrated that FLT3LG was expressed in OS tumors, the cause of circulating FLT3LG elevation and the role of FLT3 signaling in OS need to be further delineated.

An interesting aspect of FLT3LG is that it has been implicated in transdifferentiating dendritic cells into osteoclasts in inflammatory environments by substituting for M-CSF, which is critical for osteoclast precursor proliferation and survival^{37, 38}. Osteoclast differentiation regulates bone turnover and alters the bone niche essential for bone tumor initiation and promotion³⁹. Another essential factor in osteoclast differentiation is RANKL⁴⁰, whose receptor, RANK, is associated with poor disease-free survival in OS⁴¹. Children's Oncology Group is conducting a Phase 2 clinical trial using RANKL inhibitor Denosumab for recurrent or refractory OS. In contrast, a recent study suggested that FLT3LG could negatively regulate osteoclast formation⁴². Furthermore, FLT3 signaling is important for host immune system maintenance by increasing the formation of regulatory T cells³⁶, and FLT3LG-induced killer dendritic cells produced in mice can eliminate OS cells *in vitro*⁴³. Therefore, potential therapeutic benefits of targeting FLT3-axis in OS are still unclear and need to be further explored.

Although CCL4 was excluded from our final prognostic model, it was the only biomarker that significantly discriminated OS patient from normal controls. STAT3, a transcription factor for CCL4, has been shown to promote OS metastatic progression⁴⁴. Furthermore, CCL4 has been associated with inflammation-mediated prostate and liver cancers⁴⁵, whereas higher circulating CCL4, along with CCL5, were predictive of hepatocellular tumors in patients with cirrhosis⁴⁶. Thus, circulating CCL4 may have functional and early-detection relevance in OS.

Despite the encouraging results, we recognize limitations in the current study. Although we have controlled for collection protocol, time of sample collection, and known clinical factors, there may be unknown confounding factors of outcome in this retrospective analysis. Thus, conducting a prospective biomarker study in a future clinical trial will address these limitations and provide additional prognostic validation.

We believe this study is the first to report on the prognostic significance of circulating CXCL10 and FLT3LG in OS patients. It has a high clinical impact since these biomarkers could potentially guide therapies as well. Our results may be applicable to other cancers such as melanoma, colon cancer, and breast cancer, in which CXCR3 ligands are associated with tumorigenesis and metastasis^{22, 23}, as well as other bone tumors exhibiting important bone niche roles such as Ewing sarcoma³⁹. Hence, upon further investigation, CXCL10/CXCR3 and FLT3LG/FLT3 axes may lead to biomarker-guided therapies not only for OS, but also for other types of cytokine-driven tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Kaplan–Meier analysis of significant proteins with respect to overall and event-free-survival in the OS validation cohort. Higher values of all proteins corresponded to worse prognosis, i.e. $HR > 1$. All analyses reached statistical significance ($p < 0.05$), except CCL4 in event-free-survival.

Figure 2.

Kaplan-Meier analysis of combined CXCL10/FLT3LG prognostic model. Three distinct groups corresponding to “Neither biomarkers above cutoff”, “Either CXCL10 or FLT3LG above cutoff”, and “Both biomarkers above cutoff” are stratified in (A) all OS patients (B) patients with metastasis at diagnosis and (C) patients without metastasis at diagnosis. Significance was determined using the one-tailed log-rank statistic. HR 1 and 2 refer to patients with either high CXCL10 or FLT3LG, and the patients with both high, respectively, when compared to baseline hazard where both biomarkers are low.

Figure 3.

Kaplan-Meier analysis of metastatic status at diagnosis (left) and multivariate risk model (right) including CXCL10 > cutoff, FLT3LG > cutoff, and metastatic status, in both overall- (top) and event-free-survival (bottom). For metastasis, “Low risk” and “High risk” patients correspond to those with localized and metastatic disease at diagnosis, respectively. HR 1, 2, and 3 refer to the HR for patients with 1, 2, or 3 risk factors, compared to the baseline hazard of 0 risk factors.

Figure 4.

Representative immunohistochemistry results of CXCL10 and FLT3LG on the OS tissue microarray (original magnification, $\times 40$) are shown. (A) CXCL10: A1, intensity score = 3; A2, intensity score = 1. (B) FLT3LG: B1, intensity score = 3; B2, intensity score = 1.

Table 1

Demographic and clinical characteristics of discovery and validation cohorts for Luminex analysis. *P*-values are calculated with chi-square tests. INA: Information not available. Cases with no clinical information were not used in the calculations of % and chi-square tests.

	Discovery Number (%)	Validation Number (%)	P value
Age at diagnosis			
Median; range	13; 5-18	14; 2-31	0.155
< 10	8 (22)	30 (13)	
10	29 (78)	203 (87)	
Gender			
Male	16 (43)	130 (56)	0.154
Female	21 (57)	103 (44)	
Metastasis at diagnosis			
Yes	9 (24)	78 (33)	0.269
No	28 (76)	155 (67)	
Tumor site			
Lower limbs	28 (85)	189 (82)	0.563
Upper limbs	2 (6)	27 (12)	
Non-extremities	3 (9)	15 (6)	
INA	4	2	
Chemotherapy Response			
Good	16 (46)	44 (40)	0.524
Poor	19 (54)	67 (60)	
INA	2	122	
Relapse			
Yes	17 (49)	109 (47)	0.961
No	19 (51)	124 (53)	
INA	1	0	

Cytokines/Chemokines that significantly correlated with overall survival in the discovery cohort. The proteins are arranged in ascending order of their *p*-values in validation.

Analyte	Discovery			Validation	
	HR	<i>p</i> -value	BH-adjusted <i>p</i> -value	HR	<i>p</i> -value
CXCL10	3.0398	0.0490	0.0788	1.9367	0.0007
FLT3LG	3.0965	0.0661	0.0941	1.7539	0.0046
IFNG	3.1910	0.0439	0.0774	1.5716	0.0132
CCL4	NA *	0.0063	0.0298	1.4879	0.0374
IL-17	5.1418	0.0064	0.0298	1.3137	0.0954
IL-13	4.4673	0.0126	0.0347	1.2681	0.1242
TNF alpha	5.6476	0.0045	0.0298	1.1932	0.2006
MIP-1 alpha	5.0172	0.0230	0.0501	1.2384	0.2064
FGF-2	6.7009	0.0363	0.0739	1.2169	0.2172
IL-1R alpha	4.4684	0.0144	0.0347	1.1354	0.2925
IL-1 beta	4.8597	0.0067	0.0298	1.1014	0.3219
IL-10	5.6223	0.0150	0.0347	1.0813	0.3581
IL-15	4.9846	0.0081	0.0299	1.0550	0.4128
IL-9	3.2825	0.0544	0.0839	1.0164	0.4730
TNF beta	4.7125	0.0122	0.0347	1.0024	0.4956
VEGF	6.5340	0.0399	0.0739	0.9722	0.5488
GRO	6.7203	0.0015	0.0282	0.9631	0.5615
IL-1 alpha	5.5701	0.0040	0.0298	0.9580	0.5765
MCP-3	3.5613	0.0399	0.0739	0.9084	0.6640
IL-8	12.0830	0.0027	0.0298	0.8595	0.6818
IL-4	4.8464	0.0100	0.0335	0.8762	0.7125
IL-3	3.4649	0.0609	0.0901	0.8251	0.7471
TGF alpha	7.6122	0.0003	0.0100	0.8227	0.8242
MDC	0.2477	0.0134	0.0347	1.3662	0.8357

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Analyte	Discovery			Validation	
	HR	p-value	BH-adjusted p-value	HR	p-value
IL-6	4.4548	0.0073	0.0298	0.7727	0.8855
MCP-1	6.1744	0.0473	0.0788	0.7103	0.8888

* No events on the “Low CCL4” patient group from discovery cohort.

Table 3

Survival analysis for (A) each biomarker based on the expression and the metastatic status (High-risk = concentration > cutoff or metastatic; Low-risk = concentration < cutoff or non-metastatic) and (B) multivariate model combining CXCL10, FLT3LG, and metastatic status at diagnosis.

Table 3A. Model	Survival Analysis	5-year Surv: Low-risk	5-year Surv: High-risk
CCL4	Overall	68% (58%-79%)	55% (47%-64%)
	Event-free	53% (43%-66%)	46% (38%-55%)
CXCL10	Overall	66% (59%-74%)	45% (34%-59%)
	Event-free	54% (47%-63%)	35% (25%-49%)
FLT3LG	Overall	65% (58%-72%)	43% (32%-59%)
	Event-free	54% (46%-62%)	32% (21%-48%)
IFNG	Overall	66% (58%-74%)	49% (39%-61%)
	Event-free	54% (46%-63%)	40% (30%-52%)
Metastasis	Overall	67% (60%-75%)	44% (34%-57%)
	Event-free	57% (49%-66%)	30% (21%-44%)

Table 3B. Model	Survival Analysis	5-year Surv: Low-risk	5-year Surv: Intermediate-risk	5-year Surv: High-risk	5-year Surv: Very-high-risk
CXCL10 + FLT3LG + Metastasis	Overall	77% (68%-87%)	54% (44%-65%)	47% (33%-69%)	10% (2%-62%)
	Event-free	64% (54%-75%)	47% (37%-58%)	27% (14%-52%)	0%