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Research Article

Higher Serum IL-8 is associated with Reduced PFS and OS during Systemic Therapy in Metastatic Breast, Pancreatic and Prostate Cancer: A Retrospective Study in Patients from BOLERO-2 Trial, Pancreatic and Prostate Cancer Cohorts

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Abstract

Micro Abstract: This manuscript reports circulating interleukin 8 (IL-8) biomarker from three large patient cohorts. The study evaluated the clinical utility of higher circulating IL-8 to predict reduced clinical outcome in these 3 large patient cohorts. The analysis represented a variety of common solid cancers treated with conventional cancer therapeutics. The analysis demonstrates the potential of circulating IL-8 as a biomarker of reduced outcome to various conventional and to immune checkpoint inhibitor therapies. Circulating IL-8 may therefore be an important companion biomarker to potentially select cancer patients for IL-8-targeted therapy, in combination with conventional and/or immune checkpoint inhibitor therapy. **Background:** We present results from serum IL-8 and outcomes from a large, randomized phase III clinical trial in metastatic breast cancer (BOLERO-2), and in pancreatic and prostate cancer cohorts. **Methods:** Retrospective analysis included patient samples from original phase III randomized, double-blinded, multi-center clinical trial of pancreatic cancer, double-blinded, international, phase III randomized clinical trial that included 724 post-menopausal patients who had HR+ and HER2-negative advanced breast cancer and a cohort of

patients with metastatic castration-resistant prostate cancer (mCRPC). **Results:** Higher categorical IL-8 levels (median cutpoint) were significantly prognostic for shorter OS (median OS: 17.5 vs. 39.2 months; HR=2.47) and for shorter PFS (median PFS: 2.9 vs. 6.8 months; HR=1.84) in BOLERO-2. Similarly, pretreatment serum IL-8 levels in the pancreatic cohort were significantly correlated with overall survival (OS) (HR= 1.004; p=0.012). Furthermore, higher IL-8 levels above the optimal cutpoint in prostate cancer (>23rd percentile) had a median OS of only 12.8 months, compared with IL-8 levels below this cut-point with significantly longer OS of 29.9 months (HR=2.09, p<0.0001). **Conclusion:** in the BOLERO-2 metastatic breast cancer trial, higher pretreatment serum IL-8 levels predicted for reduced response to both exemestane (hormone therapy) and everolimus (mTOR inhibitor). Similarly, higher pretreatment serum IL-8 predicted reduced response to chemotherapy in pancreatic and chemo- or hormonal therapy in prostate cancer cohorts. Since higher circulating IL-8 predicted reduced response to hormonal and conventional therapies in metastatic breast, pancreatic, and prostate cancers, the significance of serum IL-8 should be further evaluated in these patients.

Keywords: Biomarker; IL-8; breast cancer; pancreatic cancer; prostate cancer;

Abbreviations: CVs: Coefficients of variation; CBR: Clinical benefit rate; EVE: Everolimus; EXE: Exemestane (EXE); HCC: Hepatocellular carcinoma; IL-8: Interleukin-8; mCRPC: Metastatic castration-resistant prostate cancer; NSCLC: Non-small cell lung cancer; ORR: Objective response rate; OS: Overall survival; PFS: Progression free survival; PD-L1: Programmed Death Ligand-1; PSA: Prostate-specific antigen; QC: Quality Control; RCC: Renal cell carcinoma; 5-FU: 5-fluorouracil

Introduction

Interleukin 8 (IL-8) [or chemokine (C-X-C motif) ligand 8, CXCL8] is a chemokine produced by macrophages, epithelial cells, monocytes, and endothelial cells, and is a potent neutrophil chemoattractant [1, 2]. IL-8 binds to G-protein coupled chemokine receptors CXCR1 or CXCR2 and is known to mediate response to various infections, and in the pathogenesis of various cancers [1, 3]. IL-8 plays a significant role in the inflammatory response, primarily via chemotaxis of target cells leading to recruitment and activation of neutrophils [2-4]. In addition to neutrophils, IL-8 also serves as a chemotactic agent for tumor cells [5]. Major IL-8 mediated effects include stimulation of endothelial and tumor cell proliferation, angiogenesis, migration, and invasion [1, 6]. In addition, IL-8 has been shown to be a potent stimulator of breast and lung cancer osteolysis [7]. Given the important role of the aforementioned factors in promoting tumor metastasis, IL-8 over-expression can therefore influence tumor metastasis and lead to disease progression in various cancers [8-10].

Serum IL-8 levels were also positively correlated with tumor size and cancer stage in patients with melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), and hepatocellular carcinoma (HCC) [11]. In patients with melanoma treated with anti-PD-1 agents and ipilimumab, IL-8 was shown to serve as an independent biomarker to monitor tumor burden and by extension to monitor disease progression as well as response to therapy [11]. IL-8 overexpression has also been linked to disease progression in melanoma [12]. Interestingly, a large-scale study that examined the association between various biomarkers and lung cancer found that only serum IL-8 levels were associated with development of lung cancer [13]. As such, the measurement of serum IL-8 levels may predict lung cancer occurrence [14]. Moreover, pancreatic cancer patients with higher serum levels of IL-8 had tumors that were more aggressive and grew more rapidly than patients with lower serum IL-8 levels [15]. In addition, IL-8 overexpression was also significantly associated with the pathogenesis of endometrial carcinoma, and in disease progression in colon cancer [16, 17]. Overall, IL-8 has a significant role in the progression of various tumor types. In this analysis, the utility of pre-treatment serum IL-8 was evaluated as a prognostic and predictive biomarker in three different cancer settings: breast, pancreatic, and prostate.

Methods:

Clinical Trial Study Design and Study Population

The original phase III randomized, double-blinded, multi-center clinical trial of pancreatic cancer included 176 patients with advanced unresectable pancreatic ductal adenocarcinoma (PDAC) [18, 19]. The study patients were divided into two arms: one arm received octreotide and a continuous infusion of 5-fluorouracil (5-FU), while the other arm received placebo and 5-FU [19, 20]. Major inclusion criteria for the phase III study included patients being at least 18 years old, with stage III histologically, pathologically, or cytologically-confirmed unresectable exocrine pancreatic adenocarcinoma, and no prior radiation, hormonal, or chemotherapies [19, 20]. The primary objective of the study was to overall survival (OS). Secondary objectives were clinical benefit, octreotide tolerability, objective response rate (ORR), and progression-free survival (PFS) [19].

BOLERO-2 was a double-blinded, international, phase III randomized clinical trial that included 724 post-menopausal patients who had HR+ and HER2-negative advanced breast cancer (NCT00863655) [21]. Briefly, patients had either recurrence or progression on their most recent therapeutics, and were then assigned in a 2:1 ratio to either everolimus (EVE) plus exemestane (EXE) (n= 485), or placebo plus EXE (n= 239) [21]. Trial patients were treated until disease progression, withdrawal of consent, or development of toxicity [21]. The primary endpoint of the BOLERO-2 trial was radiographically determined progression-free survival [21]. The secondary endpoints of the BOLERO-2 trial included overall response rate (ORR), overall survival (OS), and clinical benefit rate (CBR) [21]. In this trial, treatment arm crossover was permitted at the time of disease progression.

Thirdly, a retrospective analysis of a cohort of patients with metastatic castration-resistant prostate cancer (mCRPC) was carried out in this report. All patients had provided informed consent to allow for analysis of clinical data for further research and investigation and had their data collected in the Dana-Farber Cancer Institute (DFCI) Prostate Clinical Research Information System [22]. Retrospective identification of patients with mCRPC at time of blood draw were identified in the DFCI Prostate Clinical Research Information System in 2008 as previously reported [22]. The study patients had to show evidence of disease progression - either with rising prostate-specific antigen (PSA) or with radiographic progression [22]. The study patients' blood was obtained between February of 1998 and July of 2006 [22]. This retrospective analysis also included a subset of mCRPC patients who were chemotherapeutically naive at the time of their blood collection. The initial DFCI 2008 CRPC cohort included 362 patients: 60 from a pilot cohort and 302 from a primary cohort [22]. 152 were then excluded since they had no evidence of metastasis, or had received chemotherapy at the time of blood draw [22]. Of the remaining 210 chemotherapy-naive mCRPC patients, 9 were excluded due to no available clinical data [22]. In total, 201 chemotherapy-naive mCRPC patients were utilized in this retrospective analysis.

IL-8 ELLA Immunoassay

Serum samples were initially thawed in cold water, and serum IL-8 levels (pg/ml) were measured using the ELLA simple plex assay as per the manufacturer's instructions (ProteinSimple, San Jose, CA). The ProteinSimple Quality Control (QC) IL-8 lyophilized proteins were reconstituted as per manufacturer's instructions, and high QC and low QC were then measured in each ELLA assay as internal controls. ProteinSimple's intra-assay and inter-assay coefficients of variation (CVs) for the IL-8 high QC were 7.6% and 11.6%, respectively. ProteinSimple's intra-assay and inter-assay coefficients of variation (CVs) for the IL-8 low QC were 5.4% and 9.8%, respectively. ProteinSimple's intra-assay and inter-assay coefficients of variation (CVs) for the PD-L1 high QC were 7.6% and 11.6%, respectively. ProteinSimple's intra-assay and inter-assay coefficients of variation (CVs) for the PD-L1 high QC were 5.4% and 9.8%, respectively.

ELLA Cartridge Preparation

The serum supernatant samples were incubated at room temperature (RT) for 60 minutes. ProteinSimple high and low QC IL-8 and PD-L1 Quality Control aliquots were also thawed in cold water, and incubated at RT for 60 minutes. IL-8 ELLA cartridges were stored at 4 C prior to immunoassay, then incubated at RT for 60 minutes before sample loading. 25 microliters of serum sample were subsequently diluted with 25 microliters of sample diluent to yield a 50-microliter sample for cartridge loading. For IL-8 cartridge loading, 50 microliters of each diluted serum sample was added to each well (except for designated wells for controls). 50 microliters of PS IL-8 QC controls (high and low) was also added to designated wells. 1 ml of Wash Buffer A was then added into all buffer inlets.

ELLA Procedure

The ELLA cartridge and its package barcodes were both scanned using the ELLA software and then placed into the ELLA cartridge holder. Sample identification for each well was entered into the ELLA data sheet, and the automated ELLA immunoassay protocol was then performed to analyze the samples.

Statistics

Progression free survival (PFS) was defined as the time from randomization to the assessment of disease progression or death from any cause in the study population. Overall survival (OS) was defined as the time from the date of randomization to the date of death from any cause. Continuous variables were compared with the Wilcoxon rank test. Categorical variables were summarized as percentages and were compared with either the chi-square test or the Fisher exact test. The Kaplan-Meier method was utilized to depict the survival experience of time-to-event variables such as OS and PFS. A Cox-proportional hazard multivariate analysis was utilized for PFS and OS.

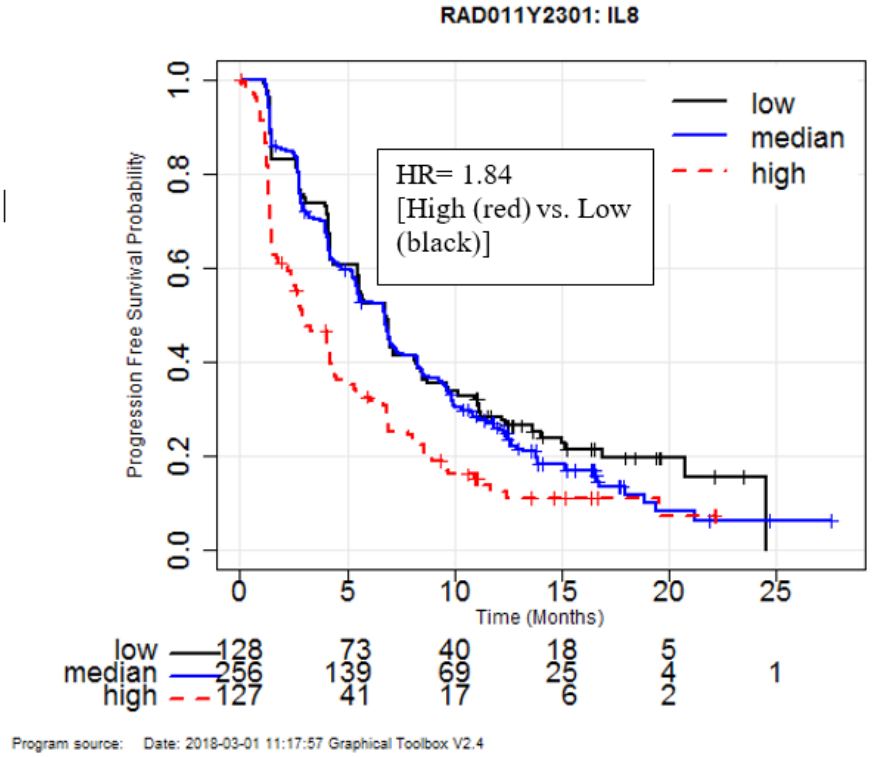
Results

In our retrospective analysis, serum IL-8 measurements of three different cancer types were determined. Firstly, the BOLERO-2 trial was an international, double-blind, phase III randomized clinical trial of 724 postmenopausal women with HR+ and HER2- advanced breast cancer who were randomized 2:1 to everolimus (EVE) + exemestane (EXE) (n=485) vs. placebo + EXE (n=239). In the original trial, the addition of EVE to EXE

resulted in significantly improved clinical outcomes (median PFS: 7.8 months vs. 3.2 months) [21]. Our retrospective biomarker analysis included 510 patients with pretreatment serum available from the BOLERO-2 trial. Results from this analysis showed that higher pretreatment serum levels of IL-8 were prognostic for significantly shorter PFS (median PFS: 2.9 months vs. 6.8 months; HR=1.84; 95% CI: 1.39 – 2.44) (Table 1) (Figure 1). Similarly, higher pretreatment serum IL-8 was also prognostic for significantly shorter OS (median OS: 17.5 months vs. 39.2 months; HR=2.47; 95% CI: 1.80 – 3.40) (Table 1) (Figure 2).

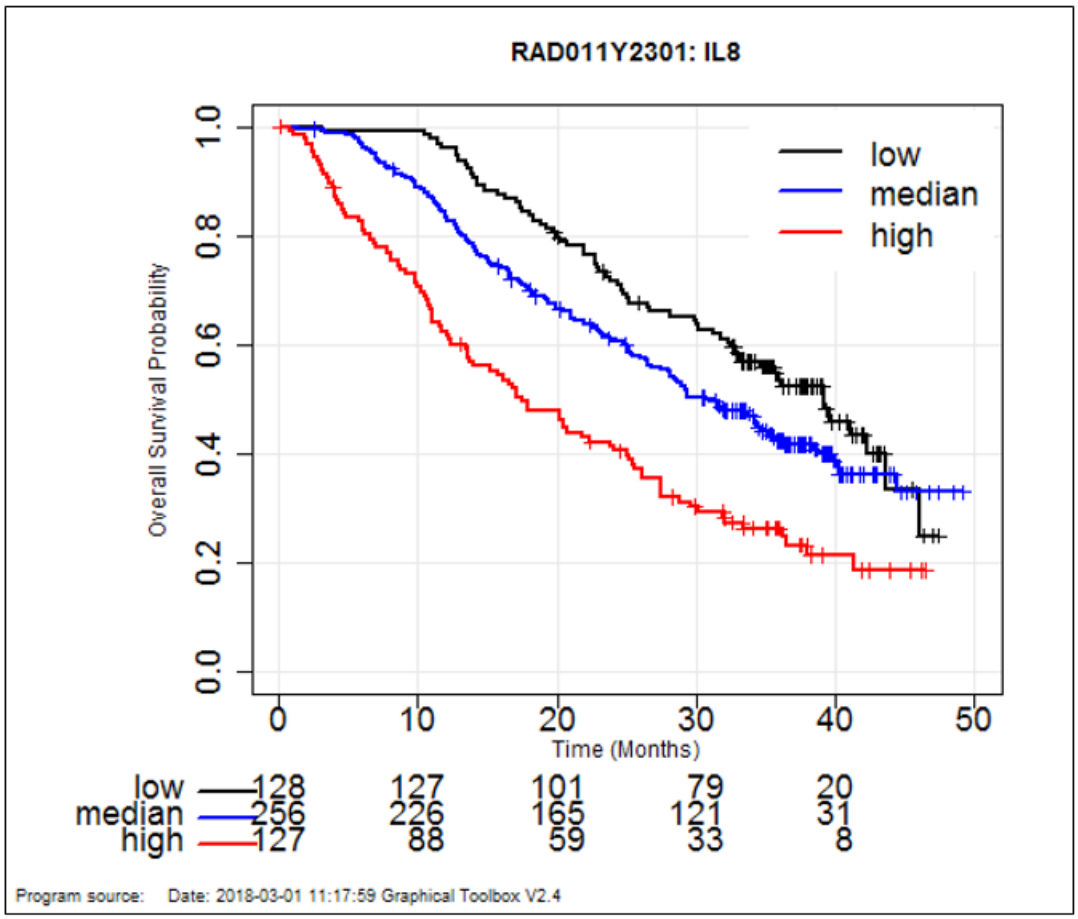
Biomarker	Serum level	N	PFS				OS			
			Events	Median (mos.)	HR*	95% CI	Events	Median (mos.)	HR*	95% CI
IL-8	Low	128	95	6.8			65	39.2		
	Middle	256	196	6.8	1.11	0.87 – 1.42	147	30.7	1.34	1.00 –1.80
	High	**127**	**104**	**2.9**	**1.84**	**1.39 – 1.84**	**94**	**17.5**	**2.47**	**1.80 -3.40**
*HR= Hazard Ratio, HR of low serum biomarker level is referent (1.0 HR).										

Table 1: PFS and OS: Serum IL-8 in metastatic breast cancer.



Group	N	Events	Median PFS (95% CI)	HR (95% CI)
low	128	95	6.8 (5.52 - 7.16)	
median	256	196	6.77 (5.42 - 7.06)	1.11 (0.87 - 1.42)
high	**127**	**104**	**2.89 (2.4 - 4.17)**	**1.84 (1.39 - 2.44)**

Figure 1: Progression-Free Survival: Serum IL-8 in metastatic breast cancer.



Group	N	Events	Median OS (95% CI)	HR (95% CI)
low	128	65	39.2 (32.85 - 43.63)	
median	256	147	30.69 (26.58 - 35.19)	1.34 (1 - 1.8)
high	**127**	**94**	**17.48 (13.54 - 23.85)**	**2.47 (1.8 - 3.39)**

Figure 2: Overall Survival: Pre-treatment serum IL-8 in metastatic breast cancer.

Secondly, the retrospective biomarker analysis from the advanced pancreatic cancer phase III clinical trial included 147 patients. In the original trial, there was no significant differences noted between the two treatment arms (5-FU vs. 5-FU + Octreotide) [18]. Kaplan-Meier life table analysis was used to correlate serum biomarkers with overall survival (OS). In univariate analysis, pretreatment serum IL-8 in our pancreatic cancer cohort was a significant biomarker as a continuous variable (HR= 1.004; p=0.012) and trended significant at the median cutpoint (HR=1.379; p=0.098) for OS. Due to multiple comparisons (in Table 2 and Figure 3), the p-values were adjusted; for table 2 and figure 3: the p-value of 0.017 for OS trended significantly different when serum PD-L1 and IL-8 levels were analyzed at the dichotomous cutpoint (medians) for both high vs. both low (HR=1.816; p= 0.017) (Table 2) (Figure 3).

Pretreatment Serum Biomarkers (median cutpoints)	# Patients	p-value^	HR	Lower 95% CI	Higher 95% CI
IL-8 low and PD-L1 low*	44 (32%)		1.0*		
IL-8 high and PD-L1 low (vs both low)	26 (19%)	0.725	1.104	0.636	1.914
PD-L1 high and IL-8 low (vs both low)	24 (18%)	0.642	1.143	0.65	2.011
Both High (vs both low)	**42 (31%)**	**0.017**	**1.816**	**1.113**	**2.963**
*Referent group; ^Multiple-testing p-value: <0.05/4; due to multiple testing the p-value of 0.017 would trend towards significant in this analysis.					

Table 2: Overall Survival (OS): Combined serum IL-8 and serum PD-L1 in advanced pancreatic cancer.

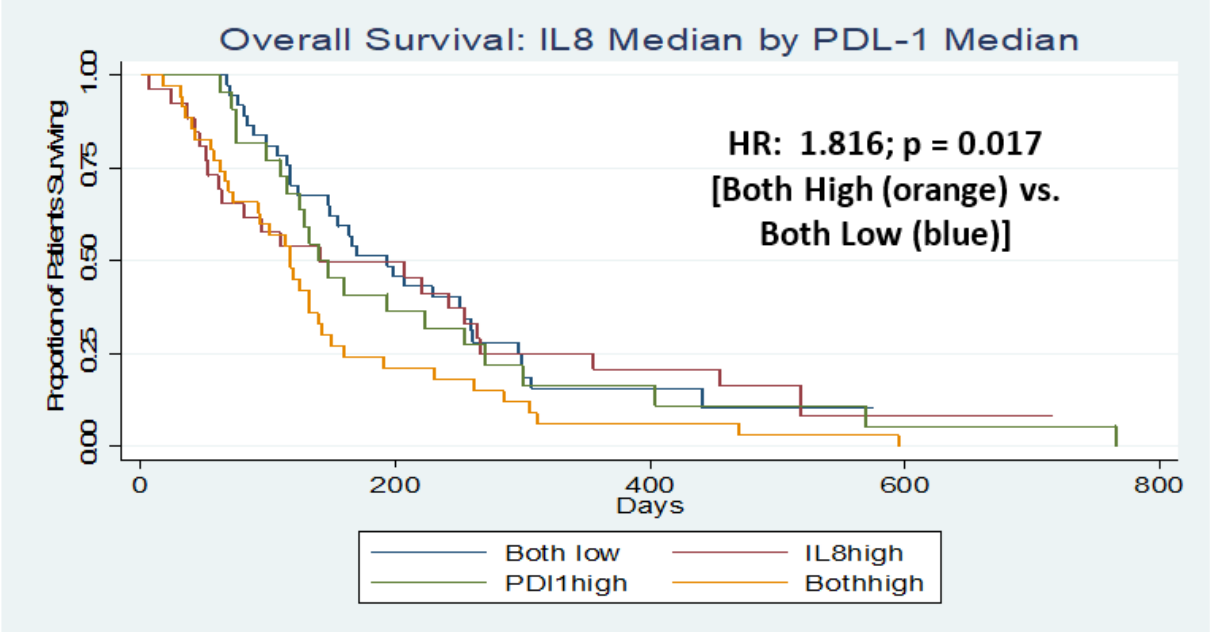


Figure 3: Overall Survival: Combined serum IL-8 and serum PD-L1 (median cutpoints) in advanced pancreatic cancer. ***** OS p-value was 0.017 trended significant in this analysis.

Thirdly, pretreatment serum IL-8 levels from 201 metastatic CRPC patients from a DFCI cohort was utilized for this retrospective analysis. Patient and disease characteristics at the time of diagnosis/blood draw are shown in Supplemental Table 1 and Supplemental Table 2. Briefly, the age at diagnosis of prostate cancer was 63 years (IQR: 63 – 77 years), approximately 35% of patients in this study had a Gleason score of 8 or higher, the median PSA at blood draw was 23.2 ng/mL (IQR: 6.7 – 78.8 ng/mL), and the time from initial diagnosis to blood draw was a median of 6.5 years (IQR: 3.0 – 10.2 years) (Supplemental Table 1). The optimal cutpoint of IL-8 in this retrospective analysis was 13 pg/mL (upper 23rd percentile), which led to the minimum p-value for OS from Cox regression (Supplemental Figure 1). Notably, pretreatment serum IL-8 levels below the upper 23rd percentile cutpoint (13 pg/mL) had a median OS of 29.9 months, whereas IL-8 levels above this cutpoint were noted to have a significantly decreased median OS to 12.8 months (HR=2.09, p<0.0001) (Supplemental Table 3, Figure 4). Similarly, pretreatment serum IL-8 above the aforementioned optimal cutpoint correlated significantly with reduced OS even when adjusted for biopsy Gleason score, age, PSA, ECOG PS, and time from initial

diagnosis to blood draw (HR=2.07, p<0.0002) (Supplemental Table 3, Supplemental Table 4). In addition, the p-value for IL-8 analyzed as a continuous variable yielded an unadjusted HR of 1.5 (1.15 - 2.03) with a p-value of 0.0033, and an adjusted HR of 1.43 (1.06 - 1.92) with a p-value 0.0187. For the adjusted continuous analysis, the following co-variates were analyzed: Gleason score (=6 ,7, =8, unknown), age, PSA (=20 & >20, unknown), and ECOG PS (0, 1+, unknown) at time of blood draw.

	N	% or Median (IQR)
At Diagnosis Biopsy Gleason		
≤6	55	27.4
7	58	28.9
≥8	70	34.8
Missing	18	9
ECOG PS at time of blood draw		
PS 0	92	45.8
PS 1	60	29.9
PS 2	8	4
Missing	41	20.4
Prior Therapy (multiple)		
Antiandrogen	186	92.5
Estrogen	28	13.9
Ketoconazole	63	31.3
Zoledronate/pamidronate	32	15.9
Age at PC diagnosis	156	63 (57-69)
Age at blood draw, year	197	71 (63-77)
PSA at blood draw, ng/mL	192	23.2 (6.7-78.8)
Time from initial diagnosis to blood draw, year	192	6.5 (3.0-10.2)

Supplemental Table 1: Patient disease status and characteristics at diagnosis/time of blood draw in metastatic prostate cancer.

	IL-8		
	Low (≤13 pg/mL) N (%)	High (>13 pg/mL) N (%)	p-value**
Total N	154	47	
At Diagnosis Biopsy Gleason*			
≤6	42 (29.8)	13 (31.0)	0.4108
7	48 (34.0)	10 (23.8)	
≥8	51 (36.2)	19 (45.2)	
PSA at blood draw, ng/mL*			
<20	70 (47.3)	12 (27.3)	0.0184

≥20	78 (52.7)	32 (72.7)	
ECOG PS*			
PS 0	79 (64.2)	13 (35.1)	0.0017
PS 1-2	44 (35.8)	24 (64.9)	
IL-8 level			
Low (≤13 pg/ml)			
High (>13 pg/ml)			
Time from initial diagnosis to blood draw, yr.Median,IQR	6.2 (3.0-10.4)	6.7 (2.0-10.1)	0.9689
*Missing values were excluded from the comparison. **Chi-sq test for categorical and Wilcoxon rank sum test for continuous variables.			

Supplemental Table 2: Patient and disease characteristics: Serum IL-8 Levels at the optimal cutpoint (13 pg/mL) in metastatic prostate cancer.

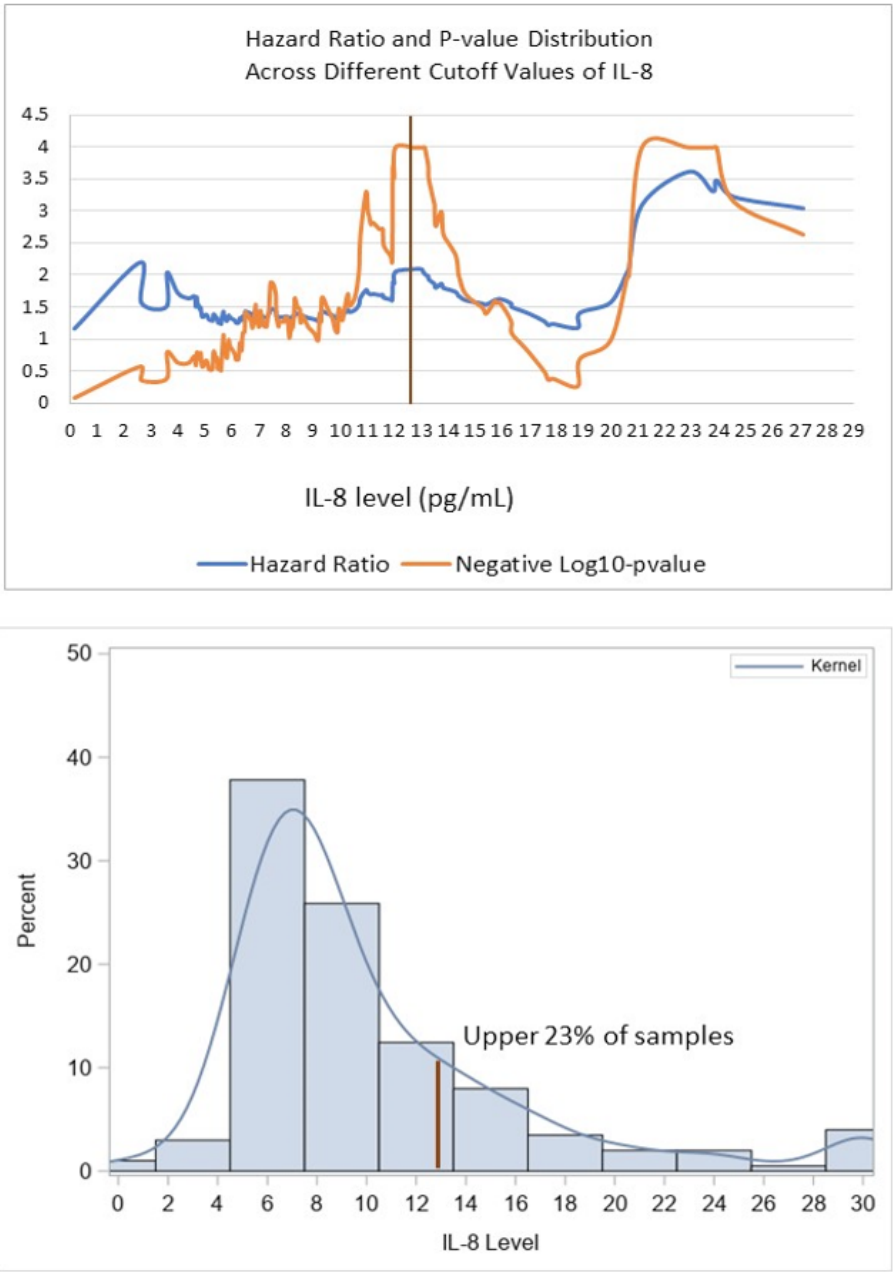
			Univariate Analysis			Multivariable Analysis	
	No. of Patients	No. of events	OS month, Median (95% CI)	Unadjusted HR (95% CI)	p-value*	Adjusted HR (95% CI)	p-value*
IL-8							
Low (≤13 pg/mL)	154	127	29.9 (24.0-36.6)	reference		reference	
High (>13 pg/mL)	**47**	**44**	**12.8 (5.8-19.6)**	**2.09 (1.48-2.96)**	**<0.0001**	**2.07 (1.42-3.01)**	**0.0002**
*Wald Chisq test from Cox regression							

Supplemental Table 3: OS: Serum IL-8 at the optimal cutpoint (13 pg/mL) in metastatic prostate cancer.

Parameter	Levels	HR	95% CI		p-value
IL8 Level (high vs. low)					
	High (>13 pg/ml)	**2.07**	**1.42**	**3.02**	**0.0002**
Time from initial diagnosis to blood draw, yr.		1	0.96	1.05	0.9865
	≤6	1 (Ref.)			
At Diagnosis Biopsy Gleason	7	0.85	0.54	1.33	0.4666
	=>8	1.44	0.92	2.26	0.107
	Missing	0.7	0.3	1.68	0.428
Age at blood draw, year	-	1.03	1.01	1.05	0.0075
	≤20 ng/mL	1 (Ref.)			
PSA at blood draw, ng/mL	>20ng/mL	2.31	1.63	3.27	<.0001
	Missing	2.5	1.12	5.61	0.0261

	0	1 (Ref.)			
ECOG PS at time of blood draw	1+	1.28	0.89	1.83	0.1872
	Missing	2.06	1.25	3.4	0.0049

Supplemental Table 4: Multivariate analysis by pretreatment serum IL8 at the optimal cutpoint (13 pg/mL) in metastatic prostate cancer.



Supplemental Figure 1: Identification of 13 pg/mL, or upper 23rd percentile, as the optimal cutpoint with minimal p-value for OS from Cox in metastatic prostate cancer. *values>30 were fixed at 30 for the graphing purpose.

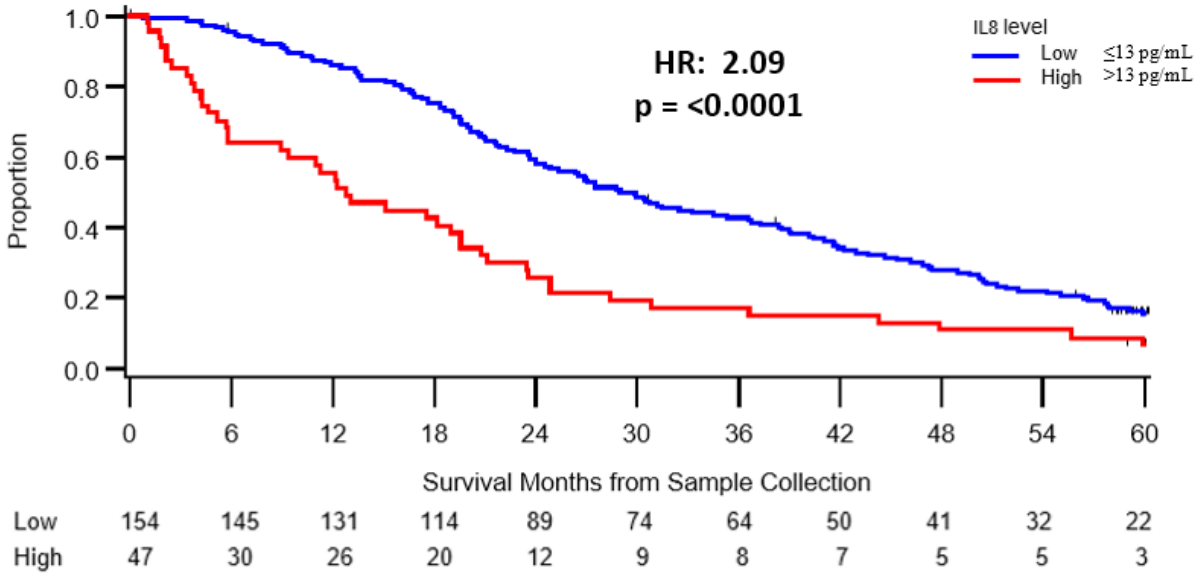


Figure 4: Overall Survival: Pre-treatment serum IL-8 levels in metastatic prostate cancer.

Discussion

These results confirm that IL-8 is a significant adverse prognostic biomarker in breast, pancreatic, and prostate cancers. Higher pretreatment serum levels IL-8 were significantly correlated with reduced OS in breast, pancreatic, and prostate cancers. Additionally, higher serum IL-8 levels were also associated with significantly reduced PFS in the BOLERO-2 phase III metastatic breast cancer trial. Findings from our multiple solid-tumor cohorts are similar to several previous studies documenting disease progression and increased tumor burden in patients with higher IL-8 levels [9-17].

An important mechanism for IL-8 mediated tumor progression has been proposed to involve the regulation of osteolysis as well as angiogenesis [23, 24]. Specifically, IL-8 induces both endothelial cell proliferation as well as capillary tube organization, effects that were blocked by monoclonal antibodies against IL-8 [25]. The combination of the IL-8-mediated enhancement of the aforementioned endothelial cell proliferation combined with the upregulation of endothelial cell-VEGF expression therefore stimulated angiogenesis [26].

Furthermore, IL-8 may also contribute to tumor progression by inducing the epithelial-to-mesenchymal transition (EMT), a hallmark feature of malignancy [27]. Results from a recent analysis showed that IL-8 secreted by tumor cells that are undergoing EMT induced adjacent epithelial tumor cells into EMT as well [27]. Complementing the aforementioned findings, results from a recent study further indicated that IL-8 is the major cytokine secreted by human monocyte U937 cells; the secreted IL-8 then led to overexpression of the adhesion molecule fibronectin, which is an essential marker of EMT, cancer invasion, and motility [28].

Interestingly, IL-8 has also been shown to activate stemness and block apoptotic processes to evade cytotoxic immune cell-mediated killing. Persistence of cancer stem cells (CSCs) is a major cause of relapse and metastasis since CSCs are often highly resistant to therapy [29, 30]. IL-8, specifically, has been shown to increase CSC phenotype. The IL-8 axis is associated with driving the CSC phenotype in several cancers, including breast [31], colon [32], and pancreatic cancers [33]. Moreover, IL-8 inhibition was associated with reduced tumor growth and stemness marker expression in colon cancer [32], and inhibition of its associated CXCR1 yielded similar depletion of breast [34] and pancreatic [33] CSCs as well as decreased tumor growth in lung cancer [35]. Thereby, IL-8-mediated tumor stemness and upregulation of the CSC phenotype have been well-documented across various tumors and these features are susceptible to therapeutic blockade of the IL-8/CXCR signaling.

The IL-8-mediated recruitment of neutrophils and myeloid-derived suppressor cells (MDSCs) to tumor beds may serve as another underlying mechanism of its role in potentiating tumor progression. A recent report demonstrated that IL-8/CXCR2 signaling is critical in tumor trafficking of MDSC, which is a major mechanism of tumor immune escape in sarcoma patients

treated with checkpoint inhibitors [36]. Upon blockade of the aforementioned signaling, the IL-8-mediated trafficking of MDSCs into the tumor microenvironment was inhibited; subsequent treatment with anti-PD-1 checkpoint inhibitors yielded significant antitumor effects and enhanced immune-mediated killing [36, 37]. Thus, blockade of IL-8 signaling in this subset of patients presents a novel strategy to improve immune checkpoint inhibitor (ICI) efficacy by decreasing MDSC recruitment at tumor sites.

A recent large-scale retrospective analysis by Schalper et al. measured baseline serum IL-8 levels from 1,344 patients who were treated with either the ICI nivolumab alone, or nivolumab plus ipilimumab in four separate phase III clinical trials (CheckMate 067-Melanoma; CheckMate 017 squamous NSCLC; CheckMate 057 non-squamous NSCLC; CheckMate 025 RCC) [38]. The analysis then assessed the objective response rate (ORR), PFS, and OS among patients in these two treatment groups. Notably, this analysis found that elevated levels of baseline serum IL-8 were associated with worse outcomes among the patients with advanced malignancies treated with ICIs (nivolumab +/- ipilimumab, everolimus or docetaxel) in the aforementioned phase III trials [38]. The study authors proposed serum IL-8 level of 23 pg/mL as a clinically meaningful cut-off, and patients in all four clinical trials receiving ICIs were stratified independently using this cut-off [38]. Interestingly, patients with baseline serum IL-8 levels >23 pg/mL were associated with shorter OS across all tumor types and treatment arms [38]. The analysis also reported a lower ORR in patients with high baseline serum IL-8 levels (>23 pg/mL) versus low (<23 pg/mL) levels across the trials and treatment arms [38]. Overall, results from this analysis validated the prognostic role of serum IL-8 across four separate cancer types from four phase III trials.

Another large-scale analysis from multiple randomized trials of 1,445 patients with metastatic urothelial carcinoma (mUC) and metastatic renal cell carcinoma (mRCC) evaluated the association between elevated IL-8 and poor treatment response to ICIs [39]. In this report, Yuen et al. analyzed circulating IL-8 levels in peripheral blood mononuclear cells and tumors in the aforementioned cancer patients being treated with ICI (atezolizumab) [39]. The study noted that patients with elevated levels of IL-8 in plasma, peripheral blood mononuclear cells, and tumors were all associated with decreased atezolizumab efficacy in the mUC and mRCC patients [39]. Conversely, patients with lower baseline IL-8 levels were associated with increased response to atezolizumab in mUC patients [39]. Similarly, mUC patients with plasma IL-8 decline during treatment exhibited improved OS upon treatment with atezolizumab compared to chemotherapy [39]. Notably, this comprehensive analysis postulates the potential benefit of on-treatment change in IL-8 levels as a potential biomarker to assess response to ICIs [39].

Conclusion

In summary, these results demonstrated that higher pretreatment serum levels of IL-8 predicted for decreased response to hormonal (exemestane) and mTOR inhibitor (everolimus) therapy in the randomized, phase III BOLERO-2 metastatic breast cancer clinical trial. Similarly, higher pretreatment serum IL-8 was associated with decreased response to hormonal or chemotherapy in prostate cancer, and to chemotherapy in pancreatic cancer. Therefore, in patients with elevated serum IL-8 levels, agents targeting IL-8 may provide additional therapeutic benefit. A recent phase I trial human monoclonal anti-IL-8 antibody (BMS-986253) has been shown to be safe and well-tolerated for use and was associated with significant decrease in serum levels of IL-8 [40]. A current study selects a cohort of higher pre-treatment serum IL-8 patients and randomizes them to nivolumab with or without BMS-986253 (monoclonal anti-IL-8 antibody) in patients with advanced cancer (NCT03400332). In conclusion, higher circulating IL-8 is a confirmed biomarker of resistance to multiple cancer therapies, including hormonal, chemotherapy, and ICIs. Using circulating IL-8 to select patients for anti-IL-8 therapy may therefore help overcome resistance to conventional therapy in multiple cancers.

Clinical Practice Points:

Circulating levels of interleukin 8 (IL-8) were confirmed as an adverse prognostic biomarker in three large independent patient cohorts. The study evaluated the clinical utility of higher circulating IL-8 to predict reduced clinical outcome in these cohorts of breast, pancreatic, and prostate cancer patients. The analysis demonstrated the potential of circulating IL-8 as a biomarker to predict reduced outcome to various conventional and targeted (everolimus, an mTOR inhibitor) therapies. Circulating IL-8 may therefore be an important companion biomarker to potentially select cancer patients for IL-8-targeted therapy, in combination with conventional and/or immune checkpoint inhibitor therapy. Future research should therefore consider circulating IL-8 an important companion biomarker to potentially select cancer patients for IL-8-targeted therapy, in combination with conventional and/or immune checkpoint inhibitor (ICI) therapy.

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Data sharing statement: Data available on request from the authors

Ethics Statement:

1. BOLERO-2 Trial:

a. Penn State Health Milton S. Hershey Medical Center (PHMC) approved protocol 10/28/2014 – Study 00001001. Our IRB did not require individual patient consent for our biomarker analysis protocol.

b. BOLERO-2 Registration Trial #: NCT00863655

2. Pancreatic CA Trial:

a. PSHMC approved protocol 8/18/2009 – HY01-273EP-A. Our IRB did not require individual patient consent for our biomarker analysis protocol.

3. Prostate CA cohort:

a. PSHMC approved protocol 2/20/2008 – 22661EP. Our IRB did not require individual patient consent for our biomarker analysis protocol.

Author contributions: The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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