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Predictive value of serum biomarkers for response of limited-stage AIDS-associated Kaposi sarcoma to antiretroviral therapy with or without concomitant chemotherapy in resource-limited settings

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Abstract

Background: Guidelines for limited-stage human immunodeficiency virus-associated Kaposi sarcoma (AIDS/KS) recommend antiretroviral treatment (ART) as initial therapy. However, many such individuals show worsening KS and require additional chemotherapy. Methods to identify such patients are lacking.

Setting: We studied whether serum levels of biomarkers associated with angiogenesis, systemic inflammation, and immune activation, which are elevated in HIV-infected individuals and implicated in the development of KS, could prospectively identify individuals with limited-stage AIDS-KS who would benefit from chemotherapy administered with ART.

Methods: Serum specimens were obtained from participants in a randomized trial evaluating the value of adding oral etoposide chemotherapy ART in treatment-naïve people with limited-stage AIDS-KS in resource-limited settings. Serum biomarkers of inflammation (CRP, IL-6, IL-8, IL-10, G-CSF, sTNFR2), immune system activation (sIL2Rα, CXCL10/IP10, CCL2/MCP1) and angiogenesis (VEGF, MMP-2, MMP-9, endoglin, HGF) were measured at entry to determine whether baseline levels are associated with KS response. On-treatment changes in biomarker levels were determined to assess how etoposide modifies the effects of ART.

Results: Pre-treatment CRP and IL-10 were higher in those whose KS progressed, and lowest in those who had good clinical responses. Pre-treatment CRP, IL-6 and sTNFR2 showed significant associations with KS progression at the week-48 primary endpoint. Immediate etoposide led to lower inflammation biomarker levels compared to ART alone. Early KS progression was associated with elevated pre-treatment levels of inflammation-associated biomarkers and increasing levels post-treatment.

Conclusions: Quantifying serum biomarkers, especially CRP, may help identify persons with AIDS-KS who would benefit from early introduction of chemotherapy in addition to ART.

Keywords

Kaposi Sarcoma; inflammation; AIDS; HIV; chemotherapy

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) is associated with increased serum concentrations of many soluble cytokines and markers of angiogenesis, systemic inflammation, and immune activation[1,2]. Excess production of these cytokines has been shown to be involved in the development of several co-morbid conditions whose incidence is increased among people living with HIV (PLWH). Among these are conditions caused by the Kaposi sarcoma herpesvirus (KSHV), which include, in addition to Kaposi sarcoma (KS), primary effusion lymphoma (PEL), multicentric Castleman's disease (MCD), several other KSHV-associated lymphoproliferations, and the KSHV-associated inflammatory cytokine syndrome (KICS). The incidence of KS is particularly high in resource-limited settings where co-infection with HIV and the Kaposi sarcoma herpesvirus (KSHV) is high[3].

Targeted treatment with anti-angiogenic, anti-inflammatory, and immunomodulatory agents has induced KS regression in some cases[4–12]. Additionally, levels of these biomarkers

may decrease during treatment with effective antiretroviral therapy (ART)[1], which may partly account for regression of KS in some individuals after ART is initiated[13,14]. Such observations have provided support for the recommendation that mild-to-moderate (i.e., limited-stage), asymptomatic or mildly symptomatic KS be treated initially with ART alone, with additional anti-KS therapy reserved for those individuals whose KS fails to respond to ART or those with more advanced, symptomatic, or life-threatening disease[15,16].

Although ART monotherapy leads to tumor regression in some individuals with HIV-associated KS, this approach is not always successful. KS may remain unchanged or, in some cases, may worsen after initiation of ART, with or without accompanying inflammatory signs and symptoms characteristic of immune reconstitution inflammatory syndrome (KS-IRIS)[17,18]. Little is known, however, about how best to predict outcomes of limited-stage KS after ART or which individuals might benefit from additional KS-directed therapy from the outset.

A5264/AMC-067, entitled “A Randomized Evaluation of Antiretroviral Therapy Alone or with Delayed Chemotherapy versus Antiretroviral Therapy with Immediate Adjunctive Chemotherapy for Treatment of Limited Stage AIDS-KS in Resource-Limited Settings (REACT-KS)”, was a prospective, randomized, open-label clinical trial conducted in sub-Saharan Africa and South America, that was designed to study the value of adding chemotherapy with oral etoposide to ART (“immediate” treatment) compared to ART alone (with delayed chemotherapy, “as needed”), in PLWH with limited-stage AIDS-KS[19]. Although the primary composite and ordinal endpoint at study week 48 did not significantly differ between the two treatment arms, time to KS progression (p=0.021), KS-IRIS (p=0.003), early KS progression (KS-PD) (P < 0.001), and KS response (p=0.003) favored the “immediate” treatment arm[19,20].

As part of this study, we assessed serum levels of cytokines and soluble markers of angiogenesis, systemic inflammation and immune activation in participants at study entry to determine whether baseline levels are associated with KS clinical outcomes and compared changes in serum levels during treatment to assess how oral etoposide modifies the effects of ART on these serum biomarkers.

MATERIALS AND METHODS

Study population and study design

Serum specimens were obtained at baseline (pre-treatment), study weeks 4, 24 and 48, and at treatment discontinuation, from participants enrolled in A5264/AMC-067 (ClinicalTrials.gov NCT01352117)[19]. Of the 190 participants originally randomized, 184 (96%) had baseline specimens available, and 175 (92%) had specimens available at study week 4 (3 participants died, 1 participant withdrew consent, and 11 participants did not have serum specimens available). Fewer participants had specimens available at study weeks 24 (n=142) and 48 (n=132) primarily because of early study closure or death; the reduced availability of specimens at these later timepoints affected only the analysis of correlations between changes in biomarkers and CD4 cell counts. 142 and 132, respectively) had specimens available at study weeks 4, 24 and 48. Briefly, participants were HIV-1-infected

adults, aged ≥18 years with biopsy-confirmed, mild-to-moderate KS, naive to chemotherapy and initiating (n=189) or reinitiating (n=1) ART. Participants were randomized to ART (tenofovir disoproxil fumarate /emtricitabine/efavirenz) alone (chemotherapy "as-needed" arm) or to ART plus up to eight cycles of oral etoposide ("immediate" arm). Participants whose KS progressed on ART alone at study week 4 or later received etoposide as part of the "as-needed" strategy.

Although the primary week-48 study endpoint was a composite endpoint incorporating the initiation of alternative KS treatments, deaths, missed visits and loss to follow-up[19], to assess the association of biomarkers with the response of KS, KS clinical response was determined post-hoc, ordered best to worst, as follows:

Good responders: Those who showed KS CR or PR within 12 weeks of study entry that lasted for at least 24 weeks;

Stable disease: Those whose KS remained stable for at least 24 weeks and who showed neither CR, nor PR, nor PD while on study; and,

Progressors: Those whose showed KS PD without a prior response or after a response that lasted less than 24 weeks.

Because some participants were not followed long enough to assess the duration of CR, PR or stable disease, they were not evaluable for the post-hoc clinical response and were excluded from the analysis.

An additional analysis was performed to assess the association of biomarkers with the response of KS assessed at the time of the primary week-48 study endpoint, and categorized the response of evaluable participants as Response, Stable or Failure as previously described[19].

Early KS-PD was defined as any KS-PD that occurred within 12 weeks of ART initiation. KS-IRIS was a subset of KS-PD that relied on the judgment of the site investigator and incorporated CD4+ and HIV RNA results[20]. Biomarker levels at baseline and week 4 were similar in participants with early KS-PD assessed as IRIS and in participants with early KS-PD not assessed to be IRIS by site investigators (data not shown). Therefore, biomarker associations with early KS-PD were assessed rather than the KS-IRIS subset.

Secondary outcomes included time to initial KS progression, and KS response. Detailed information on participant characteristics and study design has been reported elsewhere[19].

Quantification of cytokines, soluble receptors and markers of angiogenesis, inflammation and immune activation

Blood was collected in 5 mL SST or red top (no additive) tubes, which were sent at room temperature to the local AIDS Clinical Trials Group (ACTG) site processing lab within an hour. The blood was allowed to clot up to 30 minutes, then was centrifuged at 1000–1200 x g for 10 minutes and serum was collected and divided into four 0.5 mL aliquots, which were stored at −70 °C. Serum aliquots were then shipped on dry ice to the ACTG Biorepository

and were stored at −70 °C prior to shipment on dry ice to the AIDS Malignancy Consortium (AMC) Biomarker Core laboratory at University of California, Los Angeles (UCLA) for measurement of biomarkers.

Serum levels of cytokines and markers of angiogenesis (vascular endothelial growth factor [VEGF], matrix metalloproteinase [MMP]-2, MMP-9, endoglin, hepatocyte growth factor [HGF]), inflammation (C-reactive protein [CRP], interleukin [IL]-6, IL-8, IL-10, granulocyte colony stimulating factor [G-CSF], soluble tumor necrosis factor receptor-2 [sTNFR2]), and immune activation (soluble IL-2 receptor alfa [sCD25/sIL2Rα], C-X-C motif chemokine ligand 10/interferon gamma-induced protein 10 [CXCL10/IP10], C-C motif ligand 2/monocyte chemoattractant protein 1 [CCL2/MCP1]) were assessed by multiplexed immunometric assays using assay kits from R&D Systems (Minneapolis, Minneapolis, USA), according to manufacturer's directions, and a Bio-Plex 200 Luminex instrument and Bio-Plex analysis software (Bio-Rad, Hercules, California, USA), in the AMC Biomarkers Core Laboratory at UCLA, as described[19,21]. Three multiplexed assay panels were used to quantify serum levels of cytokines, chemokines, soluble receptors, and angiogenesis-associated factors: panel 1 (human IL-6, IL-8, IL-10, VEGF), panel 2 (CCL2/MCP1, sIL-2Rα/sCD25, CXCL10/IP10, endoglin, G-CSF, HGF, MMP2, MMP9, sTNFR2), panel 3 (CRP). Each assay was performed in duplicate. To monitor assay performance, duplicate quality control serum samples from a healthy adult donor were tested on each assay plate.

Statistical Methods

Baseline biomarker data and changes in biomarker data from baseline to week 4 were compared among the clinical response groups using the nonparametric Kruskal-Wallis rank test, by treatment arm ("as-needed" etoposide, "immediate" etoposide) and both arms combined. Comparisons of biomarker changes from baseline to week 4 by study arm, regardless of response, were conducted with the nonparametric Wilcoxon rank sum test.

Comparisons of baseline biomarkers and 4-week change in biomarkers for participants with and without early KS-PD were made using the Wilcoxon rank sum test. The clinical KS response was dichotomized as good responders/stable or progressors, and its association with clinical biomarkers and other baseline clinical characteristics was assessed using logistic regression models. Covariates included sex, treatment arm, KS stage, presence or absence of oral KS and edema. Numerical covariates were dichotomized as ≥ or < their median. For variable selection, each predictor of interest was first considered in a separate simple model, and $p<0.20$ determined entry into a full model. Then all-subsets variable selection approach was used with a $p<0.05$ as a criterion to retain in the final model.

Correlations between changes in biomarkers and change in CD4 at weeks 24 and 48, and between baseline HIV viral load and baseline to 4-week change in biomarkers, were assessed with the Spearman's rank correlation test.

All tests were two-sided, and *p*-values less than 0.05 were considered statistically significant. All tests were carried out with SAS software version 9.4.

No adjustments were made for multiple comparisons.

RESULTS

Study population.

Serum was available from 184 of 190 participants randomized to begin study interventions. Of these, post-hoc clinical response was determined for 177 participants: 27 good responder, 56 stable, and 94 progressors. Seven participants with baseline biomarker data were unevaluable for best response because they did not fit into one of the three possible outcome categories. Baseline characteristics were mostly similar across response groups (Table 1). The majority of participants were men in the stable group (71%) and progressors (78%), but only 44% of men were good responders (p=0.004). The median serum albumin in progressors was 3.6 g/dL, which was significantly lower (p=0.04) than in participants who were good responders and stable (3.8 g/dL).

Baseline serum CRP and IL-10 levels were significantly higher in KS progressors, and lowest in those who showed good clinical responses.

Among participants on the “as needed” arm, those who had good clinical responses had significantly lower pre-treatment serum CRP levels (median serum CRP = 2,484,350 pg/mL) than those whose KS was classified as stable (median = 5,517,150 pg/mL), or those classified as progressors (median = 12,299,000 pg/mL) (p=0.001, Table 2). Progressors had baseline CRP levels that were nearly 5-fold higher than good responders (Figure 1S). Associations of baseline biomarkers with clinical progression were less marked in the “immediate” arm (Table 1S) or both arms combined (Table 1S). For the combined group, baseline serum levels of CRP (p=0.008) and IL-10 (p=0.012) were significantly associated with KS response, with higher baseline levels seen in those who went on to have poorer clinical responses (Table 1S). Moreover, CRP levels at baseline were significantly correlated (p<0.001) with IL-6 levels at baseline (data not shown).

In simple models that evaluated the associations between clinical response and baseline characteristics, higher CD4, ANC, CRP, IL-6, sCD25 and presence of edema were associated with increased odds of progression (Table 3, simple models). Female sex and higher albumin were associated with decreased odds of being a progressor (p< 0.05). Only CRP selection led to the reduced multivariate model (Table 3, final model); high CRP was associated with higher odds (OR, 3.31; 95% CI, 1.79, 6.14) of progression.

Baseline levels of inflammation biomarkers were associated with the week 48 KS evaluation.

Baseline serum levels of CRP, IL-6 and sTNFR2 were significantly higher in participants with failure at the primary week 48 endpoint, compared to those who were stable or those with response at week 48 (Table 2S). Serum biomarker levels in the stable and response groups did not differ significantly. Additionally, no consistent patterns in biomarker changes from baseline to weeks 4, 24 or 48 were associated with the primary endpoint.

Etoposide reduces inflammation biomarkers.

Several on-treatment differences were seen between the “immediate” and “as-needed” arms. Although there was an initial increase in median serum CRP levels in both arms, participants in the “immediate” arm showed a smaller increase in CRP at week 4, and significantly greater decreases in IL-6, IL-10, sCD25, G-CSF and sTNFR2, than those in the “as-needed” arm, who had received ART only up to that timepoint (Table 4).

The development of early KS-PD was associated with elevated baseline levels of inflammation-associated biomarkers and increasing on-treatment biomarker levels.

Earlier analyses of data from this trial[19] found that early KS-PD, including KS-IRIS, was common after ART initiation, and that starting ART concurrently with etoposide reduced the incidence of both early KS-PD and KS-IRIS compared to ART alone[20]. Therefore, we compared serum biomarker levels in those persons who showed early KS-PD and those who did not. Baseline combined data from both treatment arms showed higher levels of sCD25, MMP2 and sTNFR2 in participants who developed early KS-PD than in those who did not (Table 3S).

In the “as-needed” arm, the median increase in serum CRP levels from baseline to week 4 among the participants who developed early KS-PD was nearly 7 times the median increase among those who did not (Table 5). Serum levels of sCD25 and sTNFR2 also increased in those in the “as needed” arm with early KS-PD but decreased in those without early KS-PD (Table 5). A similar picture was seen when results from both the “as needed” and “immediate” arms were combined: smaller decreases were observed in serum levels of CRP, IL-10, sCD25, and sTNFR2 from baseline to week 4 among participants with early KS-PD than among those without (Table 3S).

Baseline serum levels of inflammation-associated biomarkers showed an inverse correlation with CD4 count and a positive correlation with plasma HIV viral load.

Baseline serum levels of several inflammation-associated biomarkers (IL-6, IL-10, CCL2, IP10/CXCL10, G-CSF and sTNF-R2) showed a significant inverse correlation with baseline CD4 T cell counts (Table 4S), whereas other biomarkers (endoglin, HGF, MMP9) showed a significant positive correlation with CD4 count (Table 4S). There were also significant inverse correlations between changes in inflammation-associated biomarkers (CRP, IL-6, IL-10, sCD25, IP10/CXCL10, G-CSF, sTNFR2) and CD4 counts from baseline at weeks 24 and 48 (Table 4S).

Baseline serum levels of CRP, IL-6, IL-8, IL-10, CCL2, sCD25, IP10/CXCL10, and sTNFR2 showed significant positive correlations with plasma HIV levels (Table 4S). Similarly, a significant correlation was seen between baseline HIV plasma levels and changes in biomarkers after 4 weeks of treatment.

DISCUSSION

In this study, levels of several serum biomarkers of angiogenesis, inflammation and immune activation were measured in ART-naïve PLWH with limited-stage KS who were treated

with ART, either alone or in combination with oral etoposide[19]. Our findings suggest that pre-treatment levels of several of these biomarkers may be not only informative as predictors of clinical outcomes but also might be useful in guiding treatment. Our findings also suggest mechanisms by which etoposide decreases the risk of early KS progression and KS-IRIS.

Study participants whose KS worsened during treatment ("progressors") showed higher baseline levels of both CRP and IL-10 than those whose tumors regressed or remained stable, and higher levels of CRP were strongly predictive of subsequent KS progression in those participants on the "as-needed" arm who received ART alone without concomitant etoposide. In addition, higher baseline levels of CRP, IL-6 and sTNFR2 were associated with failure at the week-48 primary endpoint. Overall, these results are consistent with the notion that individuals whose KS responds or remains stable have lower levels of systemic inflammation prior to the initiation of treatment, and that higher levels are associated with a negative outcome. Not unexpectedly, levels of inflammation-associated serum biomarkers were also positively correlated with HIV RNA plasma levels and inversely correlated with CD4 T-cell numbers. Although HIV and KSHV infection can each cause systemic inflammation, it is noteworthy that patients in both arms of the study showed effective HIV control after initiating ART treatment, and neither baseline CD4 count nor baseline HIV viral RNA levels were associated with clinical outcomes[19]. It is possible, therefore, that persistent KSHV lytic activity contributes to the observed differences in biomarker levels and clinical outcomes.

High levels of inflammatory biomarkers could signify the presence of KSHV-associated conditions, in addition to KS, that are characterized by lytic KSHV replication and which could have influenced the outcome of KS treatment. However, we consider it unlikely that participants in this study had coexisting PEL or MCD. Participants were selected not only on the basis of having limited-stage KS, but also on their ability to tolerate cytotoxic chemotherapy in clinical settings where transfusions or hematopoietic growth factor support were not readily available. Thus, patients with poor functional status, active infections, severe anemia, neutropenia or thrombocytopenia, and laboratory evidence for significant hepatic and renal impairment were excluded from participation, and approximately two-thirds of study participants had a Karnofsky performance status of 90 or more at study entry[19]. Although a preliminary analysis of routine clinical and laboratory features of participants in this trial has suggested that coexisting KICS may negatively impact survival[22], it is noteworthy that many of the features of KICS included in that analysis (low BMI, low serum albumin, low hemoglobin, edema) were excluded from our final multivariable model of KS response and only CRP remained significant in the final model.

We had a particular interest in determining whether any of the baseline biomarkers could be helpful in predicting the development of early KS-PD, as such patients have worse clinical outcomes, and a subset of early KS-PD also have KS-IRIS[20] and might benefit from earlier intervention. Among participants with early KS-PD, higher baseline levels of multiple inflammatory and/or immune activation biomarkers were associated with early treatment failure. Additionally, after 4 weeks of treatment, participants with early KS-PD

showed levels of biomarkers that were consistent with increased levels of inflammation and immune activation.

Our analysis also provides convincing evidence for a rapid strengthening by etoposide of ART-induced modulation of biomarkers of inflammation and immune activation. We observed significantly greater decreases in serum levels of CRP, IL-6, IL-10, sCD25/sIL2Rα, G-CSF and sTNFR2 among participants on the "immediate" arm than in those on the "as needed" arm, a finding that concords with the observation that immediate etoposide treatment was associated with a significantly longer time to KS progression, a reduced incidence of early KS-PD, and a higher KS response rate than ART alone[19,20]. Although these improved outcomes may be ascribed to direct cytotoxic effects of etoposide on KS tumors, indirect effects of chemotherapy on inflammation and immune activation are not without precedent and have been exploited therapeutically in other diseases[23–25].

The central finding of this study, i.e., that elevated serum levels of CRP and inflammation-associated cytokines (e.g., IL-6, IL-10), and other biomarkers associated with inflammation and immune activation (e.g., sTNFR2, sCD25/sIL2Rα), were associated with clinical outcomes, is biological plausible, as IL-6 (both human and viral) and other cytokines have been implicated in the development and growth of KS[26]. Additionally, IL-6 is the primary driver of the acute phase response and of CRP production by hepatocytes[27]. Consistent with this, there were significant correlations ($p<0.001$) between IL-6 and CRP levels at baseline, and the change from baseline to week 4. sTNFR2, the soluble receptor for TNFα, is a marker for systemic inflammation and a surrogate marker for TNFα[28]. Overall, these findings may reflect higher pre-treatment levels of systemic inflammation, as well as a relative increase in inflammation post-treatment initiation, in those persons who went on to have clinical progression and/or early KS-PD.

The marked effect of immediate treatment with etoposide on dampening serum levels of CRP and other inflammation-associated biomarkers, combined with the much lower incidence of early KS-PD in those receiving immediate etoposide with ART[19,20], suggest that inclusion of chemotherapy in initial treatment for AIDS-KS is indicated for those persons with KS who are at high risk for rapid clinical progression, such as those who have extremely high serum levels of CRP at treatment initiation. CRP is more readily detectable in serum/plasma than cytokines, including IL-6 and IL-10; median levels of cytokines (typically <50 pg/ml) are often near the limit of detection of high-sensitivity immunometric assays, whereas median CRP levels (typically >50,000 pg/ml) are much higher, and therefore easier to measure accurately. Additionally, the increase in serum CRP levels in acute-phase responses can be as great as 10,000-fold[27,29], a much greater relative increase than is seen in serum levels of inflammation-associated cytokines, which rarely increase by more than 100-fold. While KSHV activity is likely the proximal cause of both the elevated levels of both inflammatory cytokines and CRP, and elevated KSHV viral loads have been associated with prognosis in patients with KS[30,31], most clinical laboratories can quantify CRP whereas quantification of KSHV and cytokine levels is not widely available in settings where the burden of KS is highest and access to chemotherapy is limited. Thus, quantification of CRP prior to treatment initiation may be useful in identifying patients, particularly those in resource-limited settings, who might benefit from the early addition

of chemotherapy to ART. It is important to note that the CRP levels detected in most participants in this study appeared to be higher than the levels typically seen in healthy adult persons[27,29], but considerably lower than those reported by Polizzotto et al[32] in their description of the clinical and laboratory features of KICS. Since CRP levels are elevated in most individuals with untreated HIV infection, further studies will be needed to set an optimal CRP cut-off that can be used, possibly together with other clinical and laboratory features, as a tool to identify individuals most in need of immediate chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Demographic Characteristics of Study Participants at Baseline by Clinical Response

	Good Responder (n=27)	Stable (n=56)	Progressors (n=94)	*p*-value
Age (years), median (Q1, Q3)	33.0 (30.0, 41.0)	34.0 (28.5, 42.5)	34.0 (29.0, 40.0)	0.922
Sex (female), n (%)	15 (55.6)	16 (28.6)	21 (22.3)	0.004
Ethnicity				0.365
Hispanic or Latino	4 (14.8)	4 (7.1)	5 (5.3)	
Not Hispanic or Latino	23 (85.2)	51 (91.1)	85 (90.4)	
Unknown/prefer not to answer	0 (0.0)	1 (1.8)	4 (4.3)	
Race, n (%)				0.111
Black or African American	23 (85.2)	52 (92.9)	91 (96.8)	
White	2 (7.4)	3 (5.4)	1 (1.1)	
Asian	0 (0.0)	1 (1.8)	0 (0.0)	
Other	2 (7.4)	0 (0.0)	2 (2.1)	
Country, n (%)				0.129
Brazil	2 (7.4)	3 (5.4)	3 (3.2)	
Kenya	8 (29.6)	6 (10.7)	13 (13.8)	
Malawi	8 (29.6)	20 (35.7)	30 (31.9)	
Peru	2 (7.4)	0 (0.0)	1 (1.1)	
South Africa	1 (3.7)	4 (7.1)	7 (7.5)	
Uganda	3 (11.1)	20 (35.7)	31 (33.0)	
Zimbabwe	3 (11.1)	3 (5.4)	9 (9.6)	
Weight (kg), median (Q1, Q3)	61.2 (55.0, 70.0)	61.0 (56.2, 66.4)	61.0 (53.5, 69.0)	0.711
Body mass index (kg/m^2), median (Q1, Q3)	22.7 (20.9, 26.4)	21.8 (20.5, 23.6)	21.7 (19.3, 23.4)	0.163
HIV Viral Load (log_{10}copies/ml), median (Q1, Q3)	5.1 (4.5, 5.5)	5.1 (4.7, 5.6)	5.0 (4.4, 5.5)	0.663
Hemoglobin (g/dL), median (Q1, Q3)	11.9 (10.7, 13.2)	12.2 (11.0, 13.7)	11.7 (10.4, 13.2)	0.371
Albumin (g/dL), median (Q1, Q3)	3.8 (3.2, 4.1)	3.8 (3.5, 4.0)	3.6 (3.1, 3.9)	0.040
CD4+ cell count (per mm^3), median (Q1, Q3)	180 (60, 326)	144 (68, 255)	207 (91, 330)	0.266
Neutrophils (per mm^3), median (Q1, Q3)	2090 (1340, 2930)	1715 (1230, 2725)	2030 (1430, 2840)	0.340
KS stage T0, n (%)	12 (44.4)	25 (44.6)	34 (36.2)	0.523
Oral KS present, n (%)	14 (51.9)	29 (51.8)	53 (56.4)	0.830
Edema present, n (%)	13 (48.2)	30 (53.6)	59 (62.8)	0.303

*Note: 7 participants with baseline biomarker data were unevaluable for response

Table 2:

Descriptive Statistics for Baseline Biomarkers by Clinical Response in the As-Needed Arm.

Biomarker†	Good Responder	Stable	Progressors	p-value*
As-Needed Arm, N	12	24	50	
CRP	2484350 (1197900, 11545275)	5517150 (2931500, 8475975)	12399000 (5735150, 29609500)	0.001
IL-6	2.7 (2.0, 5.0)	2.4 (1.5, 3.7)	3.2 (2.3, 8.9)	0.114
IL-8	16.8 (10.3, 21.8)	22.2 (16.0, 43.1)	15.4 (11.7, 31.1)	0.190
IL-10	1.7 (1.5, 7.5)	5.2 (2.2, 12.1)	5.6 (1.8, 20.3)	0.240
VEGF-A	106.6 (54.6, 134.1)	78.1 (46.2, 107.7)	94.0 (40.7, 154.3)	0.653
CCL-2	138.0 (121.6, 214.7)	159.0 (112.1, 255.8)	168.3 (135.7, 254.9)	0.471
sCD25/IL-2RA	1125.4 (816.2, 1328.4)	1277.0 (841.8, 1652.6)	1312.2 (888.3, 2099.2)	0.533
CXCL10/IP-10	200.3 (107.8, 345.2)	160.5 (130.1, 246.6)	138.3 (96.2, 215.6)	0.400
Endoglin	2543.4 (1766.2, 3041.4)	2474.4 (2087.8, 2826.6)	2484.2 (1811.8, 2827.9)	0.984
G-CSF	33.1 (23.0, 71.4)	44.5 (18.3, 62.9)	41.0 (13.0, 61.7)	0.925
HGF	207.8 (134.1, 314.8)	208.9 (146.4, 251.2)	226.4 (163.8, 293.4)	0.392
MMP2	84232.7 (79388.7, 90446.9)	78854.3 (71595.4, 91116.6)	82137.9 (77538.2, 90213.8)	0.264
MMP9	22713.8 (19218.0, 54513.9)	37292.9 (20401.5, 52949.6)	37431.5 (21435.7, 50728.5)	0.687
sTNFR2	5272.7 (3600.5, 7389.2)	5562.6 (3790.4, 7832.0)	5388.4 (3907.9, 8722.7)	0.885

*Nonparametric Kruskal-Wallis test.

†All values are in pg/mL, median (Q1, Q3)

Note: 7 participants (6 in as-needed arm) with baseline biomarker data were unevaluable for response

Table 3:

Associations Between Baseline Characteristics and Dichotomized Clinical Response (Good Responder/Stable vs Progressor)

		Simple Model		Full Model		Final Model	
	Covariate[a]	**OR (95% CI)**	***p*-value**[*]	**OR (95% CI)**	***p*-value**[*]	**OR (95% CI)**	***p*-value**[*]
Age (years)	≥34 vs <34	1.26 (0.70, 2.28)	0.442				
Sex	Female vs male	0.48 (0.25, 0.93)	0.030	0.57 (0.28,1.17)	0.128		
Treatment arm	As-needed vs Immediate	1.48 (0.82, 2.69)	0.193				
Weight (kg)	≥61 vs <61	0.97 (0.54, 1.75)	0.921				
Body mass index (kg/m^2)	≥22 vs <22	0.95 (0.53, 1.72)	0.872				
Hemoglobin (g/dL)	≥12 vs <12	0.74 (0.41, 1.34)	0.316				
Albumin (g/dL)	≥3.6 vs <3.6	0.44 (0.24, 0.81)	0.008	0.49 (0.23, 1.03)	0.059		
CD4+ cell count (per mm^3)	≥188 vs <188	1.77 (0.98, 3.22)	0.060	1.78 (0.90, 3.52)	0.095		
ANC (per mm^3)	≥1856 vs <1856	1.55 (0.86, 2.81)	0.149	1.20 (0.62, 2.32)	0.589		
KS stage	T1 vs T0	1.42 (0.78, 2.60)	0.255				
Oral KS present	Yes vs no	1.20 (0.67, 2.18)	0.542				
Edema present	Yes vs no	1.64 (0.90, 3.00)	0.107	1.38 (0.71, 2.69)	0.348		
CRP (pg/mL)	≥7551200 vs <7551200	3.31 (1.79, 6.14)	<0.001	2.92 (1.36, 6.24)	0.006	3.31 (1.79, 6.14)	<0.001
IL-6 (pg/mL)	≥3.2 vs <3.2	1.63 (0.90, 2.95)	0.109	0.77 (0.34, 1.72)	0.518		
sCD25/IL-2RA (pg/mL)	≥1.2 vs <1.2	1.96 (1.08, 3.57)	0.028	1.20 (0.60, 2.41)	0.608		
HGF (pg/mL)	≥195 vs <195	1.23 (0.68, 2.22)	0.495				
sTNFR2 (pg/mL)	≥5606 vs <5606	1.42 (0.79, 2.57)	0.245				

[*] Estimates are from logistic regression models.

[a] The latter group is the reference group.

ORs>1 favor the reference group.

Table 4:

Descriptive Statistics for Change in Biomarkers from Baseline to Week 4 by Treatment Arm

Biomarker†	As-Needed N=85	Immediate N=90	*p*-value*
CRP	3550350 (−18150, 13244400)	887475 (−7969500, 6244000)	0.044
IL-6	0.5 (−1.0, 2.6)	−0.5 (−2.7, 0.5)	<0.001
IL-8	1.5 (−6.7, 12.2)	0.4 (−8.1, 13.7)	0.898
IL-10	1.3 (−0.6, 7.4)	−0.3 (−2.9, 1.0)	<0.001
VEGF-A	11.2 (−7.0, 25.9)	6.6 (−16.7, 31.2)	0.302
CCL-2	−14.1 (−53.7, 19.9)	−25.7 (−66.2, 28.5)	0.501
sCD25/IL-2RA	−83.7 (−262.2, 195.7)	−301.9 (−587.5, −89.9)	<0.001
CXCL10/IP-10	−32.9 (−91.3, 2.4)	−44.8 (−108.3, −6.5)	0.254
Endoglin	245.5 (−69.7, 631.9)	196.7 (−136.1, 457.2)	0.103
G-CSF	0.0 (−29.5, 22.0)	−12.5 (−37.5, 8.5)	0.034
HGF	42.1 (−22.3, 107.1)	16.2 (−35.9, 61.1)	0.171
MMP2	2557.6 (−4447.4, 7396.0)	104.0 (−4732.0, 5556.1)	0.222
MMP9	6915.2 (−4581.1, 32260.9)	7272.5 (−3166.5, 28508.0)	0.943
sTNFR2	−206.9 (−1339.4, 959.6)	−1285.7 (−2517.9, −345.5)	<0.001

*Nonparametric Wilcoxon rank sum test.

†All values are in pg/mL, median (Q1, Q3)

Negative values indicate a decrease at week 4 compared to baseline.

Positive values indicate an increase at week 4 compared to baseline.

Table 5:

Descriptive Statistics for Change in Biomarkers from Baseline to Week 4 by Early KS-PD Status

Biomarker†	Early KS-PD		*p*-value*
	Yes	No	
As-Needed Arm, N	31	54	
CRP	7155350 (1412800, 41902000)	1149164 (−1268100, 8022800)	0.019
IL-6	0.6 (−8.5, 3.8)	0.3 (−0.8, 2.6)	0.960
IL-8	0.2 (−6.9, 9.9)	1.7 (−6.0, 13.9)	0.345
IL-10	2.4 (−0.5, 17.0)	0.8 (−0.9, 6.6)	0.331
VEGF-A	20.6 (−3.3, 45.2)	7.1 (−10.2, 22.2)	0.077
CCL-2	−.4 (−33.5, 18.8)	−16.5 (−57.5, 26.5)	0.820
sCD25/IL-2RA	74.7 (−204.6, 474.1)	−139.2 (−313.2, 70.7)	0.036
CXCL10/IP-10	−18.0 (67.1, 33.7)	−56.2 (−109.2, −3.4)	0.066
Endoglin	261.9 (−123.9, 622.3)	203.3 (−11.7, 683.2)	0.795
G-CSF	0.0 (−29.5, 41.1)	0.0 (−33.0, 17.9)	0.429
HGF	50.4 (−15.8, 149.0)	17.4 (−25.6, 72.3)	0.148
MMP2	2610.4 (−3306.9, 9083.3)	1636.2 (−4749.9, 7383.0)	0.491
MMP9	6915.2 (−4581.1, 26162.4)	6583.7 (−5589.5, 33319.5)	0.880
sTNFR2	299.9 (−821.3, 3191.9)	−511.4 (−1789.8, 802.9)	0.050
Immediate Arm, N	15	75	
CRP	3323500 (−3925000, 8262250)	486953 (−8314050, 6244000)	0.439
IL-6	−0.5 (−2.9, 0.4)	−0.6 (−2.7, 0.6)	0.970
IL-8	1.6 (−15.1, 11.4)	0.2 (−7.5, 14.2)	0.901
IL-10	−0.1 (−3.4, 12.7)	−0.4 (−2.9, 0.7)	0.286
VEGF-A	14.8 (−13.4, 35.2)	6.2 (−21.4, 31.2)	0.433
CCL-2	−16.2 (−45.9, 28.5)	−26.3 (−88.7, 29.2)	0.548
sCD25/IL-2RA	−249.0 (−572.4, 627.3)	−305.0 (−606.9, 125.9)	0.148
CXCL10/IP-10	−61.9 (−99.4, −1.7)	−43.8 (−109.0, −6.5)	0.749
Endoglin	262.5 (0.0, 550.0)	171.7 (−181.3, 446.2)	0.198
G-CSF	−5.2 (−58.0, 8.5)	−14.0 (−37.5, 9.9)	0.914
HGF	39.0 (−29.9, 92.5)	13.3 (−39.3, 53.7)	0.344
MMP2	653.0 (−4732.0, 9761.9)	−12.7 (−4932.6, 5494.0)	0.638
MMP9	3558.9 (−5712.5, 33633.4)	8796.0 (−3166.5, 28508.0)	0.693
sTNFR2	−1124.7 (−1962.2, 719.5)	−1292.0 (−2555.5, −372.4)	0.433

*Nonparametric Wilcoxon rank sum test.

†All values are in pg/mL