Abbreviated Title: Tocilizumab in KSHV-MCD
CC Protocol #: 11-C-0233 F
Version Date: 5/30/2019
NCT Number: NCT01441063

Pilot Study of Tocilizumab in Patients with Symptomatic Kaposi Sarcoma Herpesvirus (KSHV) - associated Multicentric Castleman Disease

NCI Principal Investigator: Robert Yarchoan, M.D, [A-F]
HAMB/CCR/NCI
10 Center Drive, 6N-106
Bethesda, MD 20892-1868
240-760-6075
robert.yarchoan@nih.gov

Investigational agents: none

Commercial Agents: Tocilizumab, Zidovudine and Valganciclovir

PRÉCIS

Background:

- Kaposi sarcoma herpesvirus-associated multicentric Castleman disease (KSHV-MCD) is a rare lymphoproliferative disorder that develops predominantly in HIV-infected patients. Patients often have symptoms from interleukin-6 (IL-6)(1-4), KSHV-encoded viral IL-6 (vIL-6)(3,5-8), and other cytokines
- Goals of therapy include rapid resolution symptoms and elimination of reservoirs of KSHV-infected plasmablasts.
- Tocilizumab is a humanized anti-IL-6 receptor (gp80) antibody with activity against MCD unrelated to KSHV (KSHV-negative MCD)(9). While tocilizumab does not directly affect vIL-6 signaling or other KSHV driven pathologic processes, IL-6 overproduction plays a major role in symptoms in KSHV-MCD, and blocking IL-6 may be sufficient to treat this disorder by blocking autocrine and paracrine stimulation. Combination with zidovudine (AZT) and valganciclovir (VGC), agents that target KSHV replication, have virus-activated cytotoxic activity(10), and are active in KSHV-MCD(11) may be useful and necessary in some patients.

Objective:

- Primary objective: Estimate clinical benefit of tocilizumab 8mg/kg every 2 weeks for up to 12 weeks in patients with symptomatic KSHV-MCD using a modified KSHV-MCD Clinical Benefit Response Criteria

Eligibility

- Pathologically confirmed KSHV-associated MCD
- Age ≥ 18
- At least one clinical symptom and at least one laboratory attributable to KSHV-MCD
- ECOG performance status ≤ 2
- No life- or organ-threatening manifestations of MCD
- Patients requiring therapy for rheumatoid arthritis will be excluded
- HIV-infected patients must agree to continue or start combination antiretroviral therapy

Design:

- Open label, single center pilot study. Eligible patients receive tocilizumab 8 mg/kg every 2 weeks for up to 12 weeks. In addition, patients requiring treatment intensification also receive AZT 600 mg orally q6 hours and VGC 900 mg orally q12 hours on days 1-5 of a 14-day cycle.
- Sample size 17: two stage phase II design, $\alpha=\beta=0.10$, ruling out <20% KSHV-MCD Clinical Benefit Partial Response or better with tocilizumab and targeting a >50% KSHV-MCD Clinical Benefit Partial Response or better requires 10 in the first stage. 0-2 of 10 major response: stop accrual, 3+/10: accrual to 17 total.
- Responses evaluated by KSHV-MCD Clinical Benefit Response Criteria and NCI KSHV-MCD criteria under prospective evaluation.

- Safety and tolerability evaluated using current CTCAE.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

1.1.1.1 Estimate clinical benefit of tocilizumab 8mg/kg every 2 weeks for up to 12 weeks in patients with symptomatic KSHV-MCD using a modified KSHV-MCD Clinical Benefit Response Criteria (**Appendix 1**)

1.1.2 Secondary Objectives

1.1.2.1 Evaluate best clinical, biochemical, radiographic, and overall response in patients with KSHV- associated MCD treated for up to 12 weeks with tocilizumab 8mg/kg every 2 weeks using the prior NCI KSHV-MCD Response Criteria (**Appendix 2**).

1.1.2.2 In patients with inadequate response to tocilizumab monotherapy: explore preliminarily the activity of tocilizumab 8mg/kg every 2 weeks, combined with zidovudine (AZT) 600 mg orally q6 hours (every 6 hours) and valganciclovir (VGC) 900 mg orally q12 hours (every 12 hours) on days 1-5 of a 14-day cycle.

1.1.2.3 Evaluate safety and tolerability of tocilizumab alone and in combination with AZT/VGC in this patient population

1.1.2.4 Evaluate the effect of tocilizumab on the pharmacokinetics of antiretroviral agents that are CYP3A4 substrates in patients with symptomatic KSHV-MCD

1.1.2.5 Evaluate of effect of therapy on KS using modified ACTG response criteria

1.1.2.6 Evaluate progression-free and overall survival with tocilizumab and tocilizumab/AZT/VGC.

1.2 BACKGROUND AND RATIONALE

Kaposi sarcoma herpesvirus-associated multicentric Castleman disease (KSHV-MCD)(12) is a rare lymphoproliferative disorder characterized by severe inflammatory symptoms and a waxing and waning course that is eventually lethal if untreated. It arises predominantly in HIV-infected patients. There is no standard therapy. Goals of therapy include rapid resolution of interleukin-6 (IL-6)(1-4) associated symptoms and elimination of reservoirs of pathogenic KSHV-infected cells.

Rituximab has emerged as an effective agent in the treatment KSHV-MCD(1), but can be associated with a worsening of KS(13,14). The pathophysiology of this deleterious effect is unknown, but may be due to the adverse effects of B-cell depletion(15-17) on antigen presentation or KSHV-specific antibodies(18,19). The risk for additional rare but serious infectious complications persist beyond the actual dosing of rituximab(20,21). The mechanism of action of rituximab in KSHV-MCD is unknown. The pathogenic cells of interest in KSHV-MCD are KSHV-infected, vIL-6 expressing, λ-restricted plasmablasts(22,23) predominantly in the mantle zones of lymph nodes. The KSHV infected cells themselves are generally CD20 negative(24,25); however, CD20 cells are frequently noted in KSHV uninfected lymphocytes within KSHV-MCD lymph node specimens(26) (personal observation, Uldrick, Pittaluga, Yarchoan, **Figure 1**), and it is likely that depletion of CD20 + cells with the use of rituximab

results in diminution of autocrine and paracrine signaling in the tumor microenvironment from human IL-6 and other cytokines).(27-32)

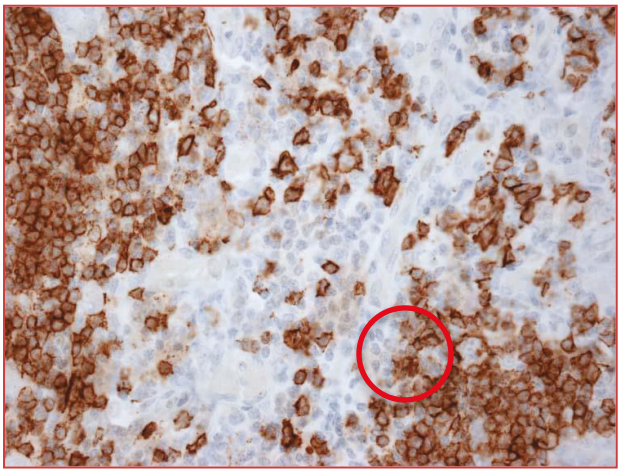


Figure 1. CD20 immunohistochemistry (brown), high power field of mantle zone of a lymph node in a patient with symptomatic KSHV-MCD. No evidence of CD20+ KSHV infected cells noted, CD20 negative plasmablasts, circled in red. Slide from S. Pittaluga.

Like MCD in patients not infected with KSHV, inflammatory symptoms and laboratory abnormalities are associated with marked increases in IL-6(3,4). Unique to KSHV-MCD, a KSHV encoded viral homologue, vIL-6(3,6-8) can be detected in the serum of most patients and likely contributes to disease pathogenesis and symptoms. Human IL-6 signal transduction requires IL-6 to first bind a co-receptor that may be either membrane bound or soluble, IL-6 receptor (gp80). The IL-6/gp80 complex can then bind gp130, leading to dimerization and intracellular signal transduction. Interestingly, vIL-6 can signal directly through gp130 without gp80(33-40). IL-6 dysregulation in KSHV-MCD(1,3,4,41) (11)is pronounced, and comparable to IL-6 dysregulation in other forms of Castleman disease; in 33 clinically defined MCD flares evaluated in the HAMB natural history study, IL-6 was elevated in 29 (88%), and the median IL-6 during flares was 24.2 pg/mL (range 1.4-171.5; p=0.0034 compared with remission)(42). These IL-6 levels are comparable with those seen in non-KSHV associated MCD (34.8 +/- 34.5 pg/mL)(9), and substantially higher than circulating IL-6 in healthy individuals (2.3 +/- 1.1 pg/mL)(43). Both IL-6 and vIL-6 are believed to contribute to disease symptomatology. Within sheets of plasmablasts arising in the setting of KSHV-associated MCD, most KSHV infected cells either moderately or strongly express the human IL-6 receptor (Figure 2F, below), and targeting pathologic IL-6 signaling through the use of a humanized monoclonal antibody for gp80 is a highly rational, B-cell sparing approach to the treatment of KSHV-MCD.

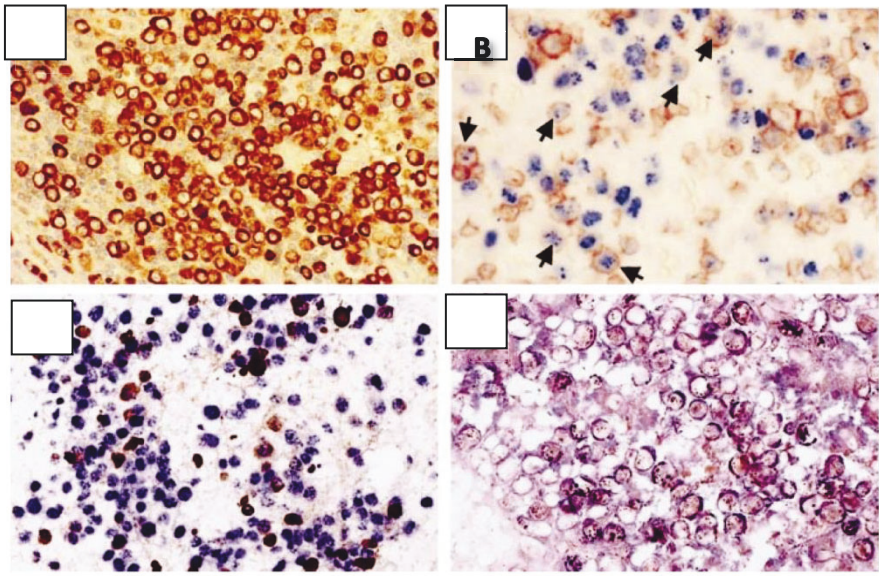


Figure 2. KSHV-positive plasmablasts in a lymphoproliferative lesion in a patient with KSHV-MCD. (A) high levels of cytoplasmic IgM (brown). (B). CD27 (brown), a marker for mature B cells, is expressed in 20% to 30% of KSHV-positive (blue) plasmablasts. (C) vIL-6 (brown) is strongly expressed in 10% to 15% KSHV-positive (blue) plasmablasts; (D) **hIL6-R (blue) is expressed in most KSHV-positive (brown) cells.** From: Du M, et. al. Blood; 2001 97(7);2130-36(23)

Anti-IL-6 therapy has been evaluated and found to be effective in both mouse models of IL-6 overproduction(44) as well as clinical studies in KSHV-uninfected patients with MCD(9,45,46). The best-studied anti-IL-6 therapy is tocilizumab, a humanized anti-IL-6 receptor (gp80) antibody, which inhibits signal transduction by both membrane bound gp80 and soluble gp80(47-49). In a phase 2 study conducted in Japan, 26 patients with KSHV-negative MCD (and 2 patients with KSHV-MCD) received tocilizumab 8 mg/kg every other week for 16 weeks, followed by an open-label extension, which resulted in improvement in symptoms, adenopathy, and laboratory abnormalities in most patients. 75% of patients on corticosteroids at baseline were able to discontinue corticosteroids(9). Tocilizumab resulted in rapid reduction in the IL-6-dependent inflammatory markers, c-reactive protein and hepcidin(50), with a gradual improvement in anemia, hypoalbuminema, lymphadenopathy, and hepatosplenomegy(9). These findings mirror the desired effects of KSHV-MCD therapy. Tocilizumab is approved in Japan for the treatment of KSHV-negative MCD. The two patients with KSHV-MCD were also reported to have responded , but no details were given. Thus, while tocilizumab does not directly affect vIL-6 signaling or other KSHV driven processes that may be pathogenic in KSHV-MCD, blocking IL-6 alone may be sufficient to treat KSHV-MCD by blocking the symptoms induced by IL-6 excess as well as stopping a cycle of vIL-6 and human IL-6 autocrine and paracrine stimulation involving both KSHV infected and KSHV uninfected cells. Also, as noted above, there is some indication from the Japanese trial that the 2 KSHV-MCD patients responded to tocilizumab.

However, it is also possible that the combination of tocilizumab with KSHV-directed therapy may be necessary, at least in some patients. The HIV and AIDS Malignancy Branch (HAMB) has been evaluating selectively targeting of KSHV-MCD plasmablasts based on their expression of KSHV lytic genes.(10) One KSHV lytic gene, *ORF36*, encodes a phosphotransferase that activates ganciclovir to a toxic triphosphate moiety, and another, *ORF21,* encodes a thymidine kinase that phosphorylates AZT.(10,51,52) We previously showed that PEL cells in which KSHV was lytically activated produced increased amounts of triphosphate moieties of AZT and ganciclovir and that AZT and ganciclovir had synergistic toxicity, at doses attainable in patients, against these PEL lines with activated KSHV.(10) We hypothesized that the combination of AZT and ganciclovir would selectively target the KSHV-infected plasmablasts expressing in KSHV-MCD and have utility in the treatment of symptomatic disease. The rationale for this regimen is to selectively target MCD-KSHV cells expressing KSHV lytic genes (*ORF21* and *ORF36*) through their activation of AZT and ganciclovir to toxic moieties. Importantly, while not all KSHV-infected MCD cells that express vIL-6 express other lytic genes, targeting lytically active KSHV-infected cells would also be expected to lead to decreases in the KSHV encoded vIL-6. Additionally, these drugs have other effects on the KSHV life cycle that may be clinical relevant. In particular, ganciclovir can inhibit KSHV replication,(53,54) and VGC, an orally available pro-drug of ganciclovir alone has been reported to reduce the frequency of flares or induce a remission in 3 patients with KSHV-MCD.(55) Through its antiviral effect, ganciclovir can reduce KSHV spread to new cells, and this may contribute to its clinical activity in KSHV-MCD. However, for cells already infected with KSHV, ganciclovir blocks a relatively late step in the KSHV lytic cycle and thus would not be expected to suppress expression of vIL-6, an early lytic gene.(56,57) Also, cidofovir, another antiviral drug with activity against KSHV, has not been found to have utility in KSHV-MCD,(58) suggesting that targeting anti-KSHV replicative activity alone is generally insufficient to yield anti-MCD activity.

To explore this hypothesis, a pilot study of high-dose AZT and VGC, an orally available pro-drug of ganciclovir, was conducted in the HAMB in patients with symptomatic KSHV-MCD. In conducting this study, we also prospectively defined clinical, biochemical, and radiographic criteria to assess responses to KSHV-MCD, and piloted the use of these criteria in assessing the responses to therapy(11). A summary of the results from this study is noted in **Table 1**. Evaluation of serum vIL-6 at baseline compared to time of best clinical response in this study suggested symptom improvements with AZT/VGC are associated with decreased vIL-6 levels (Figure 3), although our findings were not statistically significant, probably due to the relatively high cut-off of the assay for vIL-6 and a limited number of patients. This study provided evidence that a regimen of AZT combined with VGC has activity in the treatment of KSHV-MCD. Our findings support the paradigm that lytically active KSHV-infected cells and associated lytic viral proteins such as vIL-6 are important in disease pathophysiology, and that KSHV lytic genes can be used to selectively target the KSHV-infected plasmablasts in this rare lymphoproliferative disorder. Nonetheless, AZT/VGC has limitations, including hematotoxicity with chronic use, and generally slow or insufficient responses in the setting of severe IL-6 associated inflammatory symptoms. Our findings suggest that addition of AZT/VGC would be a rationale addition in patients for whom tocilizumab alone was insufficient.

Table 1. Best response to treatment with AZT/VGC in 14 patients with symptomatic KSHV-MCD

Response Category	Best Response	Number (%)
Clinical Response	Complete Response	7 (50%)
	Symptom-Free Disease	3 (21%)
	Partial Response	2 (14%)
	Major Clinical Response*	12 (86%)
	Stable Disease	2 (14%)
Biochemical Response	Complete Response	3 (21%)
	Partial Response	4 (29%)
	Major Biochemical Response†	7 (50%)
	Stable Disease	6 (43%)
	Progressive Disease	1(7%)
Radiographic Response§	Complete Response	4 (29%)
	Complete Response, unconfirmed	1 (7%)
	Major Radiographic Response‡	5 (36%)
	Stable Disease	8 (67%)
Overall Response	Complete Response	3 (21%)
	Partial Response	1 (7%)
	Stable Disease	9 (64%)
	Progressive Disease	1 (7%)

Major Clinical Response = Complete Response + Symptom Free Disease + Partial Response.
† Major Biochemical Response = Complete Response + Partial Response.
‡ Major Radiographic Response = Complete Response + Complete Response unconfirmed + Partial Response.
§ One patient was not evaluable radiographically.

From: Uldrick TS, Yarchoan R, et. al. *High-dose zidovudine plus valganciclovir for Kaposi sarcoma herpesvirus-associated multicentric Castleman disease: a pilot study of virus-activated cytotoxic therapy.* Blood 2011 Jun 30;117(26):6977-86.

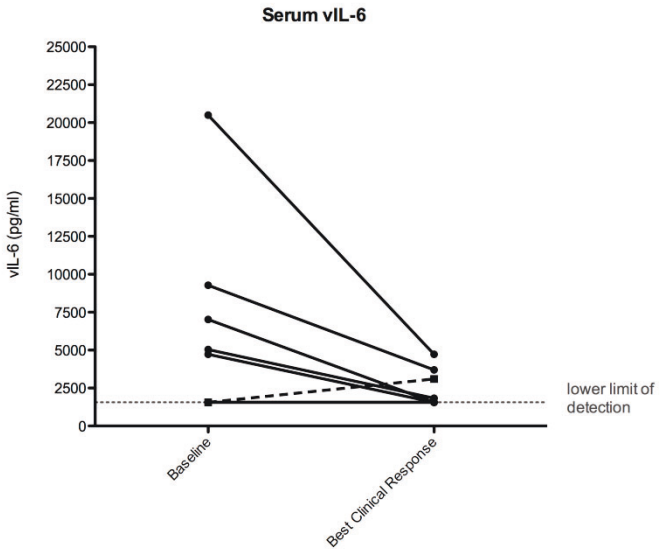


Figure 3. Changes in viral IL-6 in patients with symptomatic KSHV-MCD from baseline to time of best clinical response. Wilcoxon matched-pair signed rank test (NS) * Clinical course confounded by development endocarditis (■ - - - - ■).

The primary objective of this study is to determine the efficacy of tocilizumab in the treatment of KHSV-MCD. This may lead to less toxic therapeutic options for patients with KSHV-MCD, and will improve our understanding of disease pathogenesis. The study is designed to allow for the addition of KSHV targeted therapy with AZT/VGC should tocilizumab not provide adequate responses. Additionally, we will evaluate disease drug-drug interactions by evaluating the PK of several antiretroviral agents that are CYP3A4 substrates in patients with symptomatic KSHV-MCD, and while on tocilizumab (See Section **5.1.2**).

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Pathologically confirmed KSHV-MCD

2.1.1.2 Age ≥ 18

2.1.1.3 At least one clinical symptom probably or definitely attributed to KSHV-MCD

2.1.1.4 Intermittent or persistent fever for at least 1 week (>38°C)

2.1.1.5 Fatigue (CTCAE Grade 2 or greater)

2.1.1.6 Gastrointestinal symptoms [includes nausea and anorexia] (CTCAE Grade 1 or greater)

2.1.1.7 Respiratory symptoms [includes cough and airway hyperreactivity] (CTCAE Grade 1 or greater)

2.1.1.8 At least one laboratory abnormality probably or definitely attributed to KSHV-MCD

2.1.1.9 Anemia (Hgb [men] </=12.5 gm/dL, Hgb [women] </= 11 gm/dL)

2.1.1.10 Thrombocytopenia (</=130,000/mm^3)

2.1.1.11 Hypoalbuminemia (<3.4 g/dL)

2.1.1.12 Elevated C-reactive protein (CRP) (CRP > 3 mg/L)] probably or definitely attributable to KSHV-MCD

2.1.1.13 No life- or organ-threatening manifestations of MCD

2.1.1.14 ECOG performance status ≤ 2

2.1.1.15 HIV-infected patients should be receiving or willing to initiate an effective combination antiretroviral therapy (cART) regimen

2.1.1.16 Willingness to complete tuberculosis evaluation and start prophylactic anti-tuberculosis therapy as soon as is medically feasible if patients have a reactive tuberculin skin test and/or positive QuantiFERON-TB Gold test and have not completed an adequate course of prevented anti-tuberculosis therapy, following American Thoracic Society / Centers for Disease Control recommended guidelines: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5231a4.htm and http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm?s_cid=rr5905a1_e

2.1.1.17 Ability to understand and willingness to give informed consent

2.1.1.18 Women of child bearing potential must agree to use birth control for the duration of the study

2.1.2 Exclusion Criteria

2.1.2.1 Uncontrolled bacterial, mycobacterial, or fungal infection

2.1.2.2 Uncontrolled intercurrent illness including, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements or ability to receive therapy.

2.1.2.3 Pregnant or lactating women

2.1.2.4 Any abnormality that would be scored as NCI CTC Grade 3 toxicity that is unrelated to HIV, its treatment, or to MCD that would preclude protocol treatment. Exceptions include:

2.1.2.4.1 Lymphopenia

2.1.2.4.2 Direct manifestations of Kaposi sarcoma or MCD

2.1.2.4.3 Direct manifestation of HIV (i.e. low CD4 count)

2.1.2.4.4 Direct manifestation of HIV therapy (i.e. Hyperbilirubinemia associated with protease inhibitors)

2.1.2.4.5 Asymptomatic hyperuricemia

2.1.2.4.6 Hypophosphatemia

2.1.2.4.7 Elevated CK attributed to exercise

2.1.2.5 Past or present history of malignant tumors other than Kaposi sarcoma unless one of the following:

2.1.2.5.1 Complete remission for ≥ 1 year from completion of therapy

2.1.2.5.2 Completely resected basal cell carcinoma

2.1.2.5.3 In situ squamous cell carcinoma of the cervix or anus

2.1.2.6 Patients with concurrent Kaposi sarcoma requiring immediate cytotoxic chemotherapy

2.1.2.7 History of tocilizumab therapy within prior 6 weeks

2.1.2.8 History of rituximab or intravenous bevacizumab therapy within six weeks

2.1.2.9 History of ≥2 allergic reaction or any grade anaphylactic reaction during prior administration of tocilizumab

2.1.3 Recruitment Strategies

Recruitment of patients from outside NIH is facilitated by multiple ongoing trials at NIH. Many patients find about NIH trials from NIH websites, such as https://clinicaltrials.gov/ or NIH social media, or from other patients through social media.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for study # 01C0129 on which screening activities will be performed.

2.2.2.1 Clinical Evaluation

- Complete medical history and review of systems
- Comprehensive physical examination
- Tuberculosis Screening by Tuberculin Skin test and/or QuantiFERON TB Gold
- Documentation of previously positive results do not need to be repeated. If no history of positive tuberculosis screening test, potential subjects will undergo tuberculosis screening following CDC/ATS guidelines.
- *Tuberculin skin test.* Patients with reactive tuberculin skin test (>5 mm induration 48-72 hours after intradermal placement of 5 TU of purified protein derivative) will be evaluated by review of systems, induced sputum for AFB stain and culture for mycobacteria, and will have either a chest X-ray or CT-scan to evaluate for evidence of disease. (see http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5231a4.htm)

- *QuantiFERON TB Gold.* Call 301-496-4433 (Microbiology Specimen Processing) to order specimen tubes and coordinate specimen processing. Patients with positive results will be evaluated by:
- Review of systems
- Induced sputum for AFB stain and culture for mycobacteria
- Either a chest X-ray or CT-scan to evaluate for evidence of disease.
- Evaluation for Kaposi sarcoma, including documentation of extent of disease (See Sections, **3.4**, **6.2**, and **12.4**). If clinically warranted, additional studies for visceral KS involvement, including but not limited to: fecal occult blood test, gastrointestinal endoscopy, bronchoscopy, and CT scans, will be performed, based on clinical evaluation of the patient.

2.2.2.2 Pathology: assessment of tumor specimen(s) for histopathology reviewed by NCI-LP pathologists.

- Hematoxylin and eosin stain
- Presence of the KSHV latency associated nuclear antigen (LANA)
- Immunohistochemical evaluation for vIL-6 (for specimens being evaluated for MCD, not required for samples being evaluated for concurrent KS)

2.2.2.3 Clinical Laboratory Data

- Acute Care, Hepatic Panel, Mineral Panel, LDH.
- C-reactive protein, high sensitivity
- CBC, diff (automated lymphocyte count), reticulocyte count
- Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab, patients with detectable Hepatitis B core Ab or surface antigen require HBV DNA Quantitative
- Hepatitis C antibody, HCV RNA Quantitative PCR
- Anti-Hepatitis A IgG
- RPR
- HIV Western blot (Any previous positive result)
- HIV viral load if HIV seropositive by Western Blot
- Urine β-hCG (women)
- Fecal Occult Blood Test

2.2.2.4 Imaging

- Computerized tomography: neck, chest, abdomen, and pelvis
- ^{18}Fluorodeoxyglucose – Positron Emission Tomography / Computerized tomography (^{18}FDG-PET/CT)

2.2.2.5 Screening for Co-enrollment in other Protocols

- Patients may be co-enrolled in 04-C-0275 and/or 01-C-0038

- Screening studies listed may also be used to determine eligibility for other HAMB protocols

2.3 REGISTRATION PROCEDURES

Each patient will be reviewed by the Principal Investigator or the Lead Associate Investigator. Once it has been determined that a patient qualifies, informed consent will be obtained from the patient.

Consenting patients will generally be co-enrolled in 04-C-0275 and/or 01-C-0038.

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov . After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.3.1 Treatment Assignment Procedures

Cohort

Number	Name	Description
1	Cohort 1	Patients with KSHV-associated MCD

Arm

Number	Name	Description
1	Arm 1	Tocilizumab, Zidovudine and Valganciclovir

Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

See Section **3.4** for schedule of evaluations.

2.4.1 Clinical Evaluation

2.4.1.1 Complete medical history

2.4.1.2 Comprehensive physical examination

2.4.1.3 Kaposi sarcoma assessment if applicable (See Sections **3.4** and **6.2**)

2.4.1.3.1 Documentation of Extent of Disease

Baseline whole body photographs will be obtained upon entry into the study. At this time, 5 lesions (hereafter called marker lesions), representative of the patient's disease and, if possible, located on separate areas of the body will be selected. These marker lesions should be lesions that have never been treated with local therapies such as radiation therapy or intralesional injections. Detailed photographs of these lesions will be obtained with a metric rule beside them. The size, color and nodularity of these lesions will be recorded. Documentation will depend on the number of lesions.

2.4.1.3.2 Patients with 50 or More KS Lesions

For patients with 50 or more lesions at entry, between 1 and 3 representative areas will be selected at baseline and these will be used for each subsequent evaluation. Representative areas are sections of the body (e.g. the back, a leg, an arm, etc.), which contain at least 20 KS lesions. The total number of lesions in these representative areas will be counted and a record made of whether they are flat or raised. If, in the course of treatment, a single lesion breaks up into 2 or more smaller lesions (whose area does not extend beyond the boundary of the initial lesion), these lesions will still be counted as single lesions for the purpose of assessing total numbers in defining a response to therapy. An attempt will be made to distribute the "marker" lesions between the representative areas and the rest of the body.

2.4.1.3.3 Patients with fewer than 50 KS lesions

For patients with less than 50 lesions at entry, the total number of lesions will be counted and a record made of whether they are flat or raised.

2.4.1.3.4 Additional studies for visceral KS involvement

Additional studies, including but not limited to, fecal occult blood test, gastrointestinal endoscopy, bronchoscopy, and CT scans, will be performed at baseline where clinically indicated, based on clinical evaluation of the patient.

2.4.2 Labs required for Evaluation of MCD Biochemical Response (Performed in Clinical Center Department of Laboratory Medicine)

2.4.2.1 Acute Care, Hepatic Panel, Mineral Panel, LDH.

2.4.2.2 Complete Blood Count with differential

2.4.2.3 C-reactive protein

2.4.2.4 Ferritin (not used in MCD response evaluation, but included as an additional marker of IL-6 excess)

2.4.3 Other clinical laboratories for safety and pharmacodynamic monitoring (Performed in Clinical Center Department of Laboratory Medicine, may use screening laboratory values if performed within 7 days of study enrollment)

2.4.3.1 CPK, amylase, lipase

2.4.3.2 Lipid panel

2.4.3.3 Quantitative immunoglobulin levels, including IgE

2.4.3.4 Serum Free Light Chains

2.4.3.5 Lymphocyte phenotype TBNK. Simultaneous CBC and automated differential must be drawn.

2.4.3.6 Urinalysis and urine microscopy, patients with ≥ 2+ proteinuria will be monitored with spot urine: creatinine ratio

2.4.3.7 Urine β-hCG (women)

2.4.4 Other clinical laboratories (Performed in Clinical Center Department of Laboratory Medicine, may use screening laboratory values if performed within 14 days of study enrollment)

2.4.4.1 Thyroid function tests (TSH, free T4)

2.4.4.2 25-OH Vitamin D, 1,25(OH^2) Vitamin D

2.4.4.3 Iron Binding Capacity, Iron, Folate, B12

2.4.4.4 Haptoglobin

2.4.4.5 Type and crossmatch, direct anti-globulin test

2.4.4.6 APTT, PT, fibrinogen, lupus anticoagulant

2.4.4.7 Erythrocyte Sedimentation Rate (ESR)

2.4.4.8 RPR

2.4.5 Viral Monitoring

2.4.5.1 HIV, HBV DNA Quantitative and HCV RNA Quantitative viral load monitoring by PCR to be performed in patients with respective infections. (Performed in Clinical Center Blood Bank may use screening if performed within 7 days of enrollment)

2.4.5.2 KSHV saliva and PBMC, to be performed in the Laboratory of Dr. Denise Whitby. Saliva is collected in 50 mL Polyprophylene Conical Tubes with scope mouthwash, blood is collected in 1 ACD tube (BV Vacutainer® Ref 364606 yellow top), each labeled with unique identifier, send via messenger to Leidos Biomedical, Inc. Building 535, Room 428A, contact phone number 301-846-1714.

2.4.6 Imaging

2.4.6.1 CT-scan: neck, chest, abdomen, and pelvis, may use results from screening CT if performed within 14 days of starting tocilizumab

2.4.6.2 ^{18}FDG-PET/CT (Baseline only), may use results from screening ^{18}FDG-PET/CT if performed within 14 days of starting tocilizumab

2.4.6.3 Research Labs (See Sections **3.4**. and **5.1**.)

2.4.6.4 Two ACD tubes (BD Vacutainer® Ref 364606 yellow top) store for plasma and cells

2.4.6.5 Two serum tubes (BD Vacutainer® Ref 367820 red top tubes)

2.4.6.6 1 sodium heparin (BD Vacutainer® Ref 367878 green top tube) store for cells

2.4.6.7 Urine IFE (BD Vacutainer® Ref 364980 yellow top)

2.5 ON STUDY ASSESSMENTS

See Section **3.4** for schedule of evaluations.

2.5.1 Clinical Evaluation

2.5.1.1 Complete medical history

2.5.1.2 Comprehensive physical examination

2.5.1.3 Kaposi sarcoma assessment if applicable (See sections **3.4** and **6.2**)

2.5.1.3.1 Documentation of Extent of Disease

Baseline whole body photographs will be obtained upon entry into the study. At this time, 5 lesions (hereafter called marker lesions), representative of the patient's disease and, if possible, located on separate areas of the body will be selected. These marker lesions should be lesions that have never been treated with local therapies such as radiation therapy or intralesional injections. Detailed photographs of these lesions will be obtained with a metric rule beside them. The size, color and nodularity of these lesions will be recorded at baseline, week 7, and week 15(+/-7 days). Documentation will depend on the number of lesions.

2.5.1.3.2 Patients with 50 or More KS Lesions

For patients with 50 or more lesions at entry, between 1 and 3 representative areas will be selected at baseline and these will be used for each subsequent evaluation. Representative areas are sections of the body (e.g. the back, a leg, an arm, etc.), which contain at least 20 KS lesions. The total number of lesions in these representative areas will be counted and a record made of whether they are flat or raised. If, in the course of treatment, a single lesion breaks up into 2 or more smaller lesions (whose area does not extend beyond the boundary of the initial lesion), these lesions will still be counted as single lesions for the purpose of assessing total numbers in defining a response to therapy. An attempt will be made to distribute the "marker" lesions between the representative areas and the rest of the body.

2.5.1.3.3 Patients with fewer than 50 KS lesions

For patients with less than 50 lesions at entry, the total number of lesions will be counted and a record made of whether they are flat or raised.

2.5.1.3.4 Additional studies for visceral KS involvement

Additional studies, including but not limited to, fecal occult blood test, gastrointestinal endoscopy, bronchoscopy, and CT scans, will be performed at entry where clinically indicated, based on clinical evaluation of the patient.

2.5.2 Labs required for Evaluation of MCD Biochemical Response (Performed in Clinical Center Department of Laboratory Medicine)

2.5.2.1 Acute Care, Hepatic Panel, Mineral Panel, LDH.

2.5.2.2 Complete Blood Count with differential

2.5.2.3 C-reactive protein

2.5.2.4 Ferritin (not used in MCD response evaluation, but included as an additional marker of IL-6 excess)

2.5.3 Other clinical laboratories for safety and pharmacodynamic monitoring (Performed in Clinical Center Department of Laboratory Medicine, may use screening laboratory values if performed within 7 days of study enrollment)

2.5.3.1 CPK, amylase, lipase

2.5.3.2 Lipid panel

2.5.3.3 Quantitative immunoglobulin levels, including IgE

2.5.3.4 Serum Free Light Chains

2.5.3.5 Lymphocyte phenotype TBNK. Simultaneous CBC and automated differential must be drawn.

2.5.3.6 Urinalysis and urine microscopy, patients with ≥ 2+ proteinuria will be monitored with spot urine: creatinine ratio

2.5.3.7 Urine β-hCG (women)

2.5.3.8 Correlative Research Laboratory Studies:

2.5.3.9 Day 1 of each cycle

2.5.3.10 Three ACD tubes (BD Vacutainer® Ref 364606 yellow top) store for plasma and cells

2.5.3.11 Two serum tubes (BD Vacutainer® Ref 367820 red top tubes) store

2.5.3.12 1 sodium heparin (BD Vacutainer® Ref 367878 green top tube) store for cells

2.5.3.13 Urine IFE (BD Vacutainer® Ref 364980 yellow top)

2.5.3.14 Pharmacokinetic Studies (Performed by the Clinical Pharmacology Program)

2.5.3.15 Baseline: 10 mL Blood in EDTA (Purple top) for DMET™ SNP analysis, one time draw only in patients consenting to SNP analysis

2.5.3.16 6mL in sodium heparin (green top tube) for PK

2.5.3.17 Cycle 1

2.5.3.18 Pre-tocilizumab

2.5.3.19 15 minutes post tocilizumab

2.5.3.20 24 hours post-tocilizumab

2.5.3.21 48 hours post-tocilizumab.

2.5.3.22 Cycles 2-6

2.5.3.23 Pre-tocilizumab

2.5.3.24 15 minutes post-tocilizumab only.

2.5.4 Clinical laboratories evaluated at Baseline and Week 12 Evaluation only (Performed in Clinical Center Department of Laboratory Medicine, may use screening laboratory values if performed within 14 days of study enrollment)

2.5.4.1 Thyroid function tests (TSH, free T4)

2.5.4.2 25-OH Vitamin D, 1,25(OH^2) Vitamin D

2.5.4.3 Iron Binding Capacity, Iron, Folate, B12

2.5.4.4 Haptoglobin

2.5.4.5 Direct anti-globulin test

2.5.4.6 APTT, PT, fibrinogen, lupus anticoagulant

2.5.4.7 Erythrocyte Sedimentation Rate (ESR)

2.5.4.8 RPR

2.5.5 Viral Monitoring

2.5.5.1 HIV, HBV DNA Quantitative and HCV RNA Quantitative viral load monitoring by PCR to be performed in patients with respective infections at baseline. (Performed in Clinical Center Blood Bank may use screening laboratory values if performed within 7 days of study enrollment)

2.5.5.2 KSHV saliva and PBMC, to be performed in the Laboratory of Dr. Denise Whitby. Saliva is collected in 50 mL Polyprophylene Conical Tubes with scope mouthwash, blood is collected in 1 ACD tube (BV Vacutainer® Ref 364606 yellow top), each labeled with unique identifier, send via messenger to Leidos Biomedical, Inc.-Frederick Building 535, Room 428A, contact phone number 301-846-1714.

2.5.6 Imaging

2.5.6.1 CT-scan: neck, chest, abdomen, and pelvis [Baseline, Cycle 4 Day 1, Cycle 6 Day 15 (+/- 1 week)]

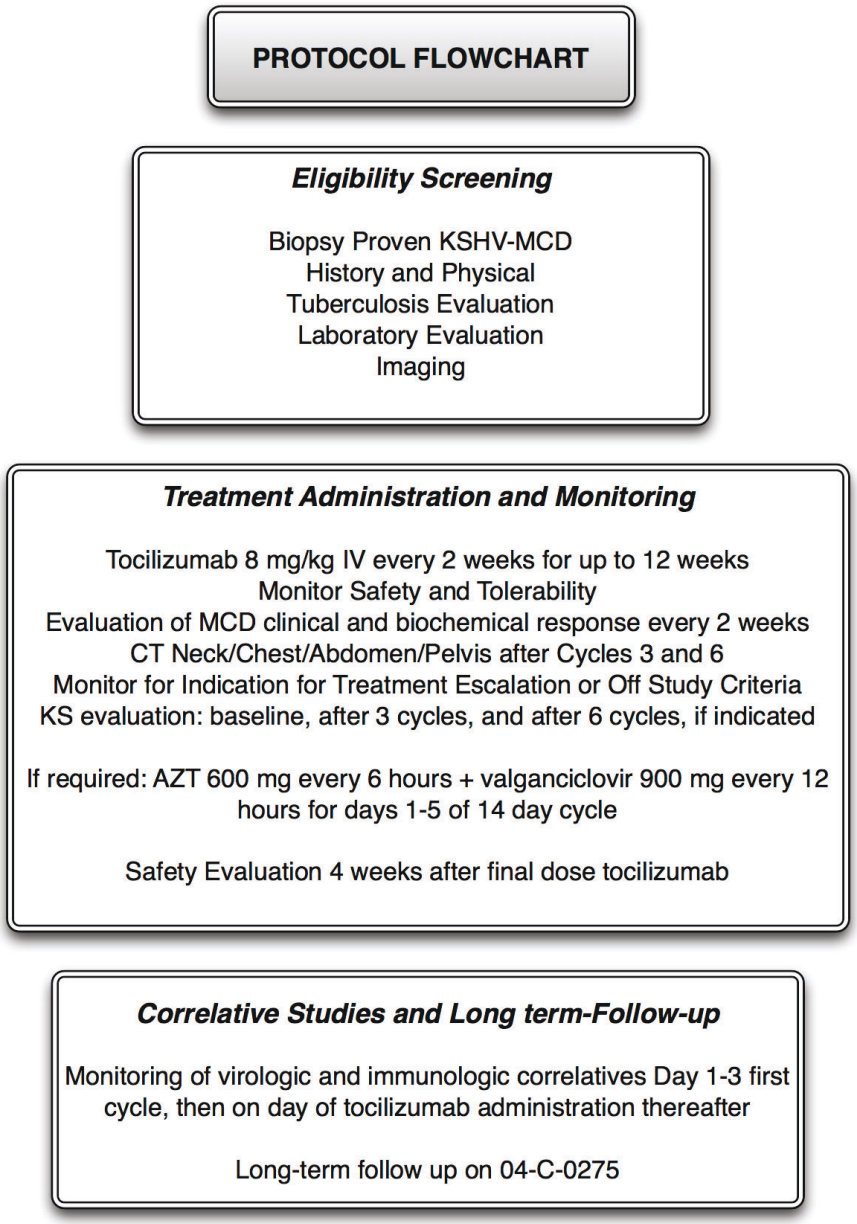
2.5.6.2 ^{18}FDG-PET/CT (Baseline only), may use results from screening ^{18}FDG-PET/CT if performed within 14 days of starting tocilizumab

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

Open label, single center pilot study.

3.1.1 Protocol Schema



3.1.2 Indications for addition of AZT/VGC

Escalation of therapy will be applied to patients having inadequate response to tocilizumab monotherapy. All decisions are based on evaluation of response using NCI KSHV-MCD Clinical Benefit Response Criteria, **Appendix 1**. Indication for addition of AZT and VGC will be based on the following criteria:

Elevated CRP (> 3.0 mg/L)(59), and one of the following:

3.1.2.1 MCD Clinical Benefit Response Criteria defined progressive disease from entry or best

response (as defined during Clinical Benefit evaluations performed at each visit using the 8 parameters in the Clinical Benefit Response Criteria) and no Off-Therapy Criteria (Section **3.6.1**)

3.1.2.2 Failure to attain improvement in any clinical or biochemical parameter probably or definitely attributed to KSHV-MCD, or presence of any grade 3 symptom probably or definitely attributed to KSHV-MCD, after week 4 (end of Cycle 2)

3.1.2.3 Failure to achieve a MCD Clinical Benefit Response Criteria defined partial response or better by end of week 8 (end of Cycle 4)

The addition of AZT/VGC will not change the total number of tocilizumab doses.

3.2 Drug Administration

3.2.1 Tocilizumab Administration

3.2.1.1 Tocilizumab 8 mg/kg, to be administered through a peripheral intravenous catheter over 60 minutes

3.2.1.2 Tocilizumab is administered on day 1 (+/- 2 days if schedule change required due to travel considerations or holidays) of a 14 day cycle

3.2.1.3 Tocilizumab to be administered for a maximum of 6 cycles, unless subject meets off-treatment criteria (Section **3.6.1**) prior to cycle 6

3.2.2 High-dose AZT and VGC Administration

If indicated (See Section 3.1.2.), AZT and VGC will be administered as follows, and continued through the final cycle of tocilizumab:

3.2.2.1 AZT and VGC will be administered concurrently with tocilizumab, with day 1 of the cycle being the day tocilizumab is administered

3.2.2.2 AZT 600 mg PO q6 hours, Days 1-5 of 14 day cycle

3.2.2.3 VGC 900 mg PO q12 hours, Days 1-5 of 14 day cycle

3.2.2.4 For inpatients not tolerating oral therapy, intravenous AZT 300 mg q6 hours and intravenous ganciclovir 5 mg/kg q12 hours may be substituted

3.3 Dose Modifications

3.3.1 Tocilizumab Monotherapy

3.3.1.1 Liver Function Abnormalities.

3.3.1.1.1 New Grade 2 AST and/or ALT (>3-5 U/L x upper limit of normal): hold tocilizumab until AST and ALT return to ≤ Grade 1 (< 3 x upper limit of normal). If recurrent Grade 2 AST or ALT abnormalities attributed to tocilizumab (not baseline abnormalities), discontinue tocilizumab

3.3.1.1.2 Grade 3 AST and/or ALT (>5 times upper limit of normal). Discontinue tocilizumab

3.3.1.2 Neutropenia

3.3.1.2.1 If neutropenia probably or definitely attributed to MCD, discuss with PI or physician clinical AI; dose adjustment may not be indicated in this setting.

3.3.1.2.2 For ANC < 0.5 K/uL not probably or definitely attributed to MCD, add filgrastim 300 mcg for patients <75 kg, 480 mcg for patients >75 kg SUBCUTANEOUS daily, and titrate subsequent frequency to maintain ANC > 1.0 K/uL, hold tocilizumab until ANC > 1.0 K/uL

3.3.1.3 Thrombocytopenia, is common in both HIV and KSHV-MCD, but has been observed occasionally in tocilizumab studies performed in patients with rheumatoid arthritis (<2%).

3.3.1.3.1 Thrombocytopenia possibly, probably, or definitely attributable to KSHV-MCD requires no dose adjustment.

3.3.1.3.2 For platelets < 50 K/uL probably, or definitely attributable to tocilizumab and unlikely related to MCD, hold tocilizumab until platelet count > 50 K/uL

3.3.1.4 Infections

3.3.1.4.1 Intercurrent uncontrolled Grade 3 infection; hold tocilizumab until ≤ Grade 2.

3.3.2 Tocilizumab combined with AZT and VGC

3.3.2.1 Intravenous administration

3.3.2.1.1 Ganciclovir 5/mg/kg IV over 60 minutes q12 hours may be substituted for oral VGC

3.3.2.1.2 Intravenous AZT 300 mg IV over 60 minutes q6 hours may be substituted for oral AZT.

3.3.2.2 Liver Function Abnormalities.

3.3.2.2.1 New Grade 2 AST and/or ALT (>3-5 U/L x upper limit of normal): hold tocilizumab, AZT and VGC until AST and ALT return to ≤ Grade 1 (< 3 x upper limit of normal). If recurrent Grade 2 AST or ALT abnormalities attributed to tocilizumab (not baseline abnormalities), discontinue tocilizumab, AZT and VGC

3.3.2.2.2 Grade 3 AST and/or ALT (>5 times upper limit of normal). Discontinue tocilizumab, AZT and VGC

3.3.2.3 Neutropenia

3.3.2.3.1 If neutropenia probably or definitely attributed to MCD, discuss with PI or physician clinical AI; dose adjustment may not be indicated in this setting.

3.3.2.3.2 For ANC < 0.5 K/uL not probably or definitely attributed to MCD, add filgrastim 300mcg for patients <75 kg, 480 mcg for patients >75 kg SUBCUTANEOUS daily, and titrate subsequent frequency to maintain ANC >1.0 K/uL.

3.3.2.3.3 Hold study drugs for up to 14 days while administering filgrastim.

3.3.2.3.4 Resume AZT and VGC at full dose once ANC >1.0 K/uL.

3.3.2.4 Anemia

3.3.2.4.1 Anemia possibly, probably, or definitely attributed to KSHV-MCD requires no dose adjustment.

3.3.2.4.2 Grade 3 anemia probably or definitely attributed to AZT and VGC (or ganciclovir): hold AZT and VGC until hemoglobin > 10 g/dL (Grade 1).

3.3.2.5 Thrombocytopenia, is common in both HIV and KSHV-MCD.

3.3.2.5.1 Thrombocytopenia possibly, probably, or definitely attributed to KSHV-MCD requires no dose adjustment.

3.3.2.5.2 For platelets < 50 K/uL probably, or definitely attributed to AZT combined with VGC and tocilizumab and unlikely related to MCD, hold study drugs until platelet count > 50 K/uL

3.3.2.6 Renal dysfunction

3.3.2.6.1 AZT: for creatinine clearance < 15 mL/min, consider 50% dose reduction.

3.3.2.6.2 VGC: adjust the dose for creatinine clearance <60 mL/min as follows (See **Table 2**):

Table 2: Dose modification of VGC in patients with renal dysfunction

Creatinine Clearance (mL/min)	Dose
40-59	450 mg twice daily
25-39	450 mg once daily
10-24	450 mg every 2 days
Hemodialysis	Use IV ganciclovir, see **Table 3**

3.3.2.6.3 Ganciclovir: adjust the dose for creatinine clearance <70 mL/min as follows:

Table 3: Dose modification of ganciclovir in patients with renal dysfunction

Creatinine Clearance	Dose (mg/kg)
50-69	2.5 every 12 hours
25-49	2.5 every 24 hours
10-24	1.25 every 24 hours
<10	1.25 three times weekly following dialysis

3.4 STUDY CALENDAR

Procedure	*Screening /Baseline*	*Cycle 1*			*Cycle 2-6*	*Week 12*	*Off Study*
		D1	*D2*	*D3*	*D1*		
History and PE[1]	X	X			X	X	X
Vital Signs	X	X	X	X	X	X	X
Biopsies[2]	X						
Tuberculosis Screen	X						
Hepatitis Evaluation[3]	X						
HIV Western blot	X						
CT Neck/Chest/Abd/Pelvis[4]	X				X[4]	X	
MCD Response Labs [5]	X	X	X	X	X	X	X
Other Labs[6]	X			X	X	X	
Baseline/Week 12 Only[7]	X					X	
Viral Monitoring[8]	X				X[8]		X
Lymphocyte TBNK	X			X	X	X	X
PD Research Specimens[9]	X			X	X	X	X
PK Research Specimens[10]	X	X	X	X	X	X	
MCD Response Evaluation	X				X	X	X
Photography /KS evaluation[11]	X				X[11]	X	
Adverse Event Monitoring	X	→					
NIH Advance Directives Form[12]	X						

1. Includes current medications, and ECOG performance status
2. Pathologic Evidence of KSHV-MCD. Kaposi sarcoma biopsy if applicable.
3. Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab, patients with detectable Hepatitis B core Ab or surface antigen require HBV –DNA viral load (PCR), Hepatitis C antibody, HCV RNA-PCR, Anti Hepatitis A IgG
4. Baseline, Day 1 Cycle 4 (+/- 1 week), documentation of possible complete response, week 12 (+/- 1 week).
5. CBC with differential, Reticulocyte count, Acute Care, Hepatic Panel, Mineral Panel, LDH, C-reactive protein, ferritin (included as marker of IL-6 induced acute phase reactant)
6. Lipid panel, quantitative immunoglobulins, IgE, free light chains, CPK, amylase, lipase, urinalysis, urine microscopy, urine β-hcg (women only). Can use screening labs if performed within 7 days of study enrollment.
7. Iron Binding Capacity, Iron, Folate, B12, PT/PTT, haptoglobin, ESR, lupus anticoagulant, TFTs, 25-OH vitamin D, Urine IFE, RPR. Can use screening labs if performed within 14 days of study enrollment.
8. HIV, HBV, and HCV in patients with evidence of infection at baseline: Baseline, Day 1 Cycle 3, Day 1, Cycle 6 (+/- one week), off study only. More frequently if clinically indicated.
9. Three yellow top tubes, three red top tubes, one green top tube, urine, saliva. (See Sections **5.1.1**, **5.2**). On Day 3, Cycle 1: 2 yellow tops, 1 red top only.
10. For all subjects: Cycle 1, Day 1: Pre-tocilizumab, 15 minutes post tocilizumab, 24 hours post-tocilizumab, 48 hours post-tocilizumab. Cycles 2-6, pre-tocilizumab and 15 minutes post-tocilizumab only. At each time point: 6mL in sodium heparin (green top tube). For patients consenting to DMET genotyping, 10 mL in EDTA at baseline.
11. If indicated, modified ACTG KS disease measurements, Day1 Cycle 1, Day 1 Cycle 4. Photography at baseline, week 12 only. Patients with clinically significant progression of KS should be documented with measurements and photography.
12. As indicated in Section **9.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

3.5 Radiation Therapy Guidelines

Radiation therapy for Kaposi Sarcoma is contraindicated.

3.6 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 4 weeks following the last dose of study therapy.

3.6.1 Criteria for removal from protocol therapy

3.6.1.1 Completion of 12 weeks of therapy

3.6.1.2 Clinical or Biochemical Progressive Disease (as compared to baseline at the point that AZT and VGC were added) on tocilizumab combined with AZT and VGC, as defined by NCI-MCD Response Criteria.

3.6.1.3 ECOG performance status ≥ 3

3.6.1.4 Life or organ threatening manifestations of KSHV-MCD including neurologic, hepatic, pulmonary, cardiovascular, or renal dysfunction equivalent to grade IV adverse event using CTCAE 4.0 probably or definitely attributed to KSHV-MCD that does not improve within 14 days of therapy (or sooner at PI discretion, see Section **3.6.1.9**)

3.6.1.5 Unacceptable toxicity at least possibly attributable to tocilizumab, AZT and/or VGC (≥ Grade 3), with exception of common abnormalities most likely due to HIV or KSHV-MCD (See Section **7.2.3**).

3.6.1.6 Patient preference

3.6.1.7 Institution of a non-protocol therapy for Kaposi sarcoma, or other malignancy (i.e. primary effusion lymphoma) diagnosed during the course of the protocol.

3.6.1.8 Pregnancy

3.6.1.9 Patients may be removed from protocol therapy, if in the opinion of the PI, the risk of continued therapy outweighs the potential benefit of continued participation or if patient non-adherence interferes with study objectives.

3.6.2 Off-Study Criteria

3.6.2.1 Completion of tocilizumab or discontinuation of tocilizumab and completion of 4 week safety follow-up visit

3.6.2.2 Patients who do not comply with follow-up visits will be taken off study

3.6.2.3 Patients with persistent toxicity at least possibly attributed to tocilizumab at the off-study visit will be monitored on study for up to 4 months after the end of therapy or until the toxicity returns to baseline, whichever occurs first, with frequency and duration of follow up based on medical need

3.6.2.4 Subjects may be taken off study at the discretion of the Principal Investigator or Lead Associated Investigator

3.6.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify the Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

Additional treatment and/or long-term follow up of patients is possible through enrollment in 04-C-0275.

4 SUPPORTIVE CARE/CONCURRENT THERAPIES

4.1 General KS and HIV Related

Supportive care medications may be administered as clinically indicated, or at the discretion of the Principal Investigator. Other specific cytotoxic or biologic therapies for KS or KSHV-MCD are prohibited, with the exception of short-term courses of corticosteroids.

4.2 Opportunistic infections

Subjects who develop opportunistic infections, including but not limited to pneumocystis jiroveci pneumonia, mycobacterial diseases, cytomegalovirus (CMV), and fungal infections will be treated using standard regimens. All opportunistic infections will be discussed with the Principal Investigator. Consultation with the Infectious Disease Service is mandatory for subjects diagnosed with mycobacterium tuberculosis. Prophylactic antibiotics are outlined in Section 4.6.

4.3 Anemia

Anemia will be treated conservatively. Transfusions will generally be avoided unless a subject develops symptomatic anemia. At the discretion of the principal investigator, transfusion may be performed for hemoglobin < 10 g/dL in patients with a history of cardiovascular disease. Appropriate evaluation for etiology of the anemia should be initiated.

4.4 Thrombocytopenia

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10 K/uL. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50 K/uL.

4.5 Febrile Neutropenia

Patients who develop febrile neutropenia will be hospitalized and treated with intravenous antibiotics. See Sections **3.3.1.2** and **3.3.2.3** for Treatment Modifications and filgrastim use pertaining to neutropenia.

4.6 CONCURRENT THERAPIES

4.6.1 Anti-retroviral Therapy

Patients will receive antiretroviral therapy if it is indicated. Combination therapy will be generally based on Department of Health and Human Services Guidelines for treatment of HIV infection, available at http://www.aidsinfo.nih.gov/guidelines/. However, patients may have extenuating circumstances requiring deviation from these guidelines. Whenever possible, changing antiretroviral therapy should be avoided unless needed for optimal patient care. Additionally, referring physicians may manage this component of patient care. In some patients who are prescribed AZT as part of the treatment for KSHV-MCD, AZT may replace another non-nucleoside reverse transcriptase inhibitor for the duration of therapy as long as there is no evidence of HIV mutations conferring resistance to AZT.

4.6.2 Anti-emetics

Patients prescribed high-dose AZT combined with VGC will receive an appropriate "as needed" anti-emetic, i.e. compazine 10 mg orally or IV every 6 hours prn. Other anti-emetic medications may be considered, however, glucocorticoids should be avoided.

4.6.3 Glucocorticoids

Glucocorticoids will not be initiated for patients on study unless indicated for a severe medical condition not attributed to KSHV-MCD. For patients on glucocorticoids at the time of enrollment, they will be tapered as soon as medically feasible. Patients on >20 mg of prednisone or equivalent for at least 3 weeks will be monitored clinically for adrenal insufficiency.

4.6.4 Pneumocystis jiroveci Prophylaxis

All HIV-infected patients with history of PCP or with CD4 cells ≤ 200/mm3 should receive Bactrim DS (sulfamethoxazole 800 mg + trimethoprim 160 mg) PO 3x/week. Alternatives include but are not limited Bactrim DS (sulfamethoxazole 800 mg + trimethoprim 160 mg) PO daily, Dapsone 100 mg PO qd, and monthly aerosolized pentamidine 300 mg. Additionally, given potential infectious risk of anti-IL-6 therapy, PCP prophylaxis is will be strongly considered during study duration in all HIV infected patients, regardless of CD4 count.

4.6.5 MAC Prophylaxis

Consider for all HIV-infected patients with a historic CD4 nadir less than 75 cells/mm^3, or for patients whose CD4 cells fall below this level while on study. Recommend azithromycin 1200 mg orally once weekly, but other agents are acceptable.

4.6.6 Latent Tuberculosis Therapy

Patients with reactive tuberculin skin test (>5 mm induration 48-72 hours after intradural placement of purified protein derivative) or positive QuantiFERON TB Gold will be evaluated by induced sputum culture for mycobacteria, and will have either a chest X-ray or CT-scan to evaluate for evidence of disease. Those who have not completed an adequate course of preventative anti-tuberculosis therapy must complete tuberculosis evaluation and start prophylactic anti-tuberculosis therapy prior to enrollment, following American Thoracic Society/ Centers for Disease Control recommended guidelines:
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5231a4.htm

Preferred regimen is isoniazid 300 mg orally daily for nine months. 10-25 mg of pyridoxine (vitamin B6) should be given orally with each isoniazid dose to reduce the risk of isoniazid-induced peripheral neuropathy.

4.6.7 Hepatitis B Virus

In patients with hepatitis B virus (HBV) and HIV, a number of HIV drugs (including but not limited to: tenofovir, emtricitabine, lamivudine) also are appropriate for HBV therapy and should be incorporated in anti-HIV regimens for HBV-co-infected patients. For HIV negative patients, HBV suppression with an appropriate anti-viral agent will be used.

4.6.8 Prevention of Herpes Simplex Virus Reactivation

Valacyclovir will be considered in patients with history of oral or genital HSV infections. Valacyclovir will not be co-administered with VGC.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Biospecimen Banking for Immunologic and Other Studies

Specimens will be collected for several planned correlative studies. Samples will be stored and run in batches. Planned assays include: viral-IL6 (performed in the laboratory of Dr. Robert Yarchoan), multiplex inflammatory cytokine assay (Cat.# K15008 and Meso-Scale Discovery, Gaithersburg, MD), soluble gp80 ELISA (Cat# 850.510.096, Gen-Probe, Cedex, France), KSHV serology, blood viral load, and saliva viral load (to be performed in the Laboratory of Dr. Denise Whitby). For all these testing, specimens will be sent via messenger to the AIDS Monitoring Laboratory (AML), Leidos Biomedical, Inc. in the NCI-Frederick. Specific assays will be performed in batches. Biospecimens for storage (See Section **5.2**) will be collected in the following tubes (See Section **3.4** for collection schedule):

5.1.1.1 Three ACD tubes (BD Vacutainer® Ref 364606 yellow top)

5.1.1.2 Two serum tubes (BD Vacutainer® Ref 367820 red top tubes)

5.1.1.3 1 sodium heparin (BD Vacutainer® Ref 367878 green top tube)

5.1.1.4 Urine IFE (BD Vacutainer® Ref 364980 yellow top

5.1.1.5 Saliva, collected in 50 mL polypropylene conical tube (Beckton-Dickenson labware) with 2 mL scope mouth wash (swish and spit)

5.1.2 Pharmacokinetic studies

5.1.2.1 Background on disease-drug-drug interactions

In the NCI Natural History study of KHSV-MCD, we have observed anecdotally that patients with active disease sometimes poorly tolerate other medications, including antiretroviral therapy. One possible explanation for this observation is that CYP3A4(60) is down regulated by IL-6(61,62) and the setting of IL-6 excess, and patients symptomatic KSHV-MCD may have higher circulating levels drugs do to alterations in metabolism(63). Furthermore, alterations in protein concentrations of albumin (decreased) and alpha 1-acid glycoprotein(64)(increased) may also effect protein binding in the setting of uncontrolled KSHV-MCD. Tocilizumab may counter

these IL-6 effects, and lead to lower drug concentrations, as has been shown for simvastatin in the setting of the treatment of rheumatoid arthritis(65).

We will analyze limited PK of commonly used antiretroviral agents (ritonovir, lopinovir, atazanavir, and efavirenz) that are metabolized by CYP450 for changes in their plasma exposure in response to tocilizumab.

5.1.2.2 Sample Collection (to be performed by the Clinical Pharmacology Program)

5.1.2.2.1 PK Studies

Patient samples will be collected as follows (also, see sections 2.4.4. and 3.4):
Cycle 1, Day 1: Pre-tocilizumab, 15 minutes post tocilizumab (i.e. 75 minutes after beginning 60 minute infusion), 24 hours post-tocilizumab, 48 hours post-tocilizumab. Cycles 2-6, pre-tocilizumab and 15 minutes post tocilizumab only. At each time point: 6mL in sodium heparin (green top tube) will be collected, for PK analysis of antiretroviral agents and tocilizumab (66). Cumulative plasma exposure of tocilizumab will be evaluated for pharmacokinetic and pharmacodynamics correlations using clinical and surrogate endpoints in Objectives **1.1.2.4**-**1.1.2.6**.
See Section **5.2.7** for specimen handling.

5.1.2.2.2 Genotyping

Genotyping of baseline samples for SNPs in drug-metabolism enzymes will be performed in the event of unexplained heterogeneity in the antiretroviral PK. Genomic DNA will be analyzed for polymorphisms in genes involved in drug metabolism and transport that have been linked to the clinical pharmacology of antiretrovirals. Genotyping will be performed using the Drug Metabolizing Enzymes and Transporters (DMET, Affymetrix) genotyping platform and results will be quality checked via direct sequencing.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.2.1 Background

It is understood that per the NCI policy regarding the Requirements for the Research Use of Stored Human Specimens and Data, prospective NIH Intramural IRB approval and continuing IRB oversight must be obtained for research involving identified or coded samples or data where investigators can identify the source. This policy applies to research protocols where the remaining research activities are limited to data analysis and to the subsequent research use of specimens or data previously collected under a now terminated protocol. The following guidelines describe how these principles apply to this specific protocol.

5.2.2 AIDS Monitoring Laboratory

Many samples on this study will be processed and stored in the AIDS Monitoring Laboratory (AML) run by Leidos Biomedical, Inc. in the NCI-Frederick facility located with Fort Detrick. The samples are stored under code, and the information linking these unique codes to the patients is kept on the AML database. The laboratory informatics system conforms to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines.

All laboratory staff is trained to adhere to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. Computers used to access inventory systems require username and password for login. The laboratory database is housed in a secure, protected environment and backups are performed routinely. Access to specimen information, clinical data, and stored specimens is limited to approved laboratory staff and the investigator in charge of the study (or individuals authorized by the investigator).

5.2.3 Specimen Withdrawal for Research Purposes

The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The research nurse on the study will be in charge of tracking this information for the protocol team.

5.2.4 Specimens Sent to the Whitby Laboratory

Some of the specimens are sent to the laboratory of Dr. Denise Whitby, also in Leidos Biomedical, Inc. in the NCI-Frederick facility located with Fort Detrick. The samples sent are coded by the protocol research team and have no patient identifiers. They are logged in by Dr. Whitby's laboratory and kept in a locked facility. They are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

Denise Whitby Ph.D.
Leidos Biomedical, Inc.
1050 Boyles St, Building 535, Room 428A
Frederick, MD
Contact phone number 301-846-1714

5.2.5 Specimens sent to the Yarchoan Laboratory

A limited number of samples will be sent to Dr. Yarchoan's laboratory from Leidos Biomedical, Inc. These will usually be sent batched. Occasional samples may be sent directly to the Yarchoan laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized.

Robert Yarchoan, M.D.
10 Center Drive, Room 5A-25
Bethesda, MD 20892
Phone Contact: 240-760-6075

5.2.6 Specimens sent to the Tosato Laboratory

A limited number of samples will be sent to Dr. Tosato's laboratory from Leidos Biomedical, Inc. These will usually be sent batched. Occasional samples may be sent directly to the Tosato laboratory. They will be kept locked and a log will be kept of the specimens and when they are utilized. The samples sent are coded by the protocol research team and have no patient identifiers. They will in general be run in batch when enough specimens are collected.

Giovanna Tosato, M.D.
Building 37, Room 4124
37 Convent Drive
Bethesda, MD 20892

240-760-6144

5.2.7 Specimens sent to the Figg Laboratory/ Blood Processing Core (BPC)

5.2.7.1 Sample Collection

PK samples will be collected in green top, sodium heparin tubes (6mL), and delivered on ice to Douglas Figg in the Blood Processing Core (BPC). Samples for genotyping of baseline SNPs in drug-metabolism enzymes (DMET) will be sent in 10mL EDTA purple top tubes.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.7.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested). The PI will report any loss or unanticipated destruction of samples per Section **7.2.1.** Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will also be reported as per Section **7.2.1**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables

5.2.8 Clinical Testing of Stored Specimens

Clinical testing of all samples will be one in accordance to the protocol. The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The research nurse on the study will be in charge of tracking this information for the protocol team.

5.2.9 Clinical Center Processing and Storage of Clinical Specimens

Many routine samples and a sample of the biopsy specimens are sent to the Laboratory of Pathology (CCR), Department of Laboratory Medicine, and Department of Transfusion Medicine at the NIH Clinical Center. These samples will be handled according to the procedures of these departments. Results for clinical testing are generally available via the CRIS electronic medical record.

5.2.10 Co-enrollment on 01-C-0038 and/or 04-C-0275

If patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples may also be tested under the specifications of that study. Similarly, if patients have co-enrolled on other studies approved by the NIH Intramural IRB that call for maintaining and testing the samples, then they may be transferred to those studies.

5.2.11 Handling of Specimens at Study Termination

At the termination of the protocol, if patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples will be transferred to that study unless the patients request that this not occur. Also, if patients have co-enrolled on other studies approved by the NIH Intramural IRB that call for maintaining the

samples, then they will be maintained on those protocols. Otherwise, the unused samples will be destroyed.

5.2.12 Loss or Destruction of Samples

The PI or LAI will report any loss or unanticipated destruction of the samples that meet expedited reporting requirements as per Section **7.2.1**, and any new use of the samples, specimens, or data will require prospective IRB approval.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through the end of treatment. At follow up visits (beyond 28 days), only adverse events which are serious and related to the study intervention need to be recorded.

Adverse events will be recorded in the database only if they occur during treatment or within 30 days of coming off treatment, unless they are assessed as being at least possibly due to study drug administration.

Any abnormal laboratory value that is specifically defined as a Grade 1 or higher Adverse Event in CTCAE version 4.0 will be recorded in the database as an AE. Abnormal laboratory values that fall into the "Investigations - Other, specify" category but are not otherwise graded will only be recorded if they are determined by the investigator to be clinically significant.

Elective hospitalizations to administer, or to simplify trial treatment or trial procedures (e.g., an overnight stay to facilitate chemotherapy and or correlative studies) are not considered serious adverse events (SAEs). However, all events leading to unplanned hospitalizations or unplanned prolongation of an elective hospitalization (e.g., undesirable effects of any administered treatment) must be documented and reported as SAEs.

6.1.1 Clinical Research Records

Members of the HIV and AIDS Malignancy Branch clinical research team will collect data on study subjects according to the Schedule of Evaluations outlined in Section **3.4**. Complete records must be maintained on each patient including supplementary information obtained from outside laboratories, radiology reports, or physician's records, as well as relevant copies of notes, reports and results from the CC – CRIS electronic medical record system. These records will serve as the primary source material that forms the basis for the research record. All research

records will be stored in a secure office of one of the clinical investigators. The primary source documentation will assure the following:

6.1.1.1 The patient satisfied each eligibility criterion.

6.1.1.2 Signed informed consent was obtained prior to registration and treatment.

6.1.1.3 Treatment was given according to protocol or any protocol violations documented and justified.

6.1.1.4 Toxicity and response were assessed according to protocol.

6.1.1.5 Drug accountability records were kept on each patient.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.1.2 Correlative and Exploratory Studies Performed with non-Clinical Collaborators

Coded identifiers will be used when collecting and storing samples or data collected in collaboration with non-clinical investigators. Management of coded identifiers for samples stored at AML is performed at AML (See Section **5.2.2**.), and management of unique identifiers in the BPC is performed at the BPC (see Section **5.2.7.**) Samples sent directly to other collaborators will be labeled with a unique identifier number and date. The study coordinator will securely maintain a log of patients and unique identification codes in the regulatory binder/file, which is only accessible to clinical investigators. (See Section **5.2**)

6.1.3 Survival Endpoints

6.1.3.1 Patients may be followed on 04-C-0275 either during, or any point after completion of this study. Data on survival endpoints related to KSHV-MCD disease progression, development additional KSHV associated malignancies, subsequent therapy and death may be collected, and used in evaluation of the long-term safety, tolerability, and efficacy of tocilizumab.

6.1.3.2 Patients not enrolled in 04-C0275 will be censored for disease progression endpoints at the time the patient is taken off-study. Overall survival data may be obtained by phone from a study subject-designated physician or durable power of attorney, or from the Social Security Death Index.

6.2 Response Criteria

In this protocol, the response criteria for the primary outcome will be the KSHV-MCD Clinical Benefit Criteria (Section **6.2.2**) The following sections outline the original criteria as defined in the KSHV-MCD Natural History Protocol 04-C-0275 (Section **6.2.1**) that will continued to be used, the newer Clinical Benefit Criteria (**6.2.2**), as well as the modified AIDS Clinical Trial Group criteria used to grade Kaposi Sarcoma responses (**6.2.3**). Also see section **8** for planned evaluation of the NCI-KSHV-MCD Response Criteria and KSHV Clinical Benefit Criteria.

6.2.1 NCI KSHV-MCD Response Criteria

6.2.1.1 Background

Response to therapy will be based on KSHV-MCD response criteria under prospective evaluation, outlined in **Appendix 1**: KSHV-MCD Clinical Benefit Response Criteriaand **Appendix 2** . The following criteria were devised in 2004, and had a minor amendment in 2008 prior to grading responses in any HAMB KSHV-MCD study. This response criteria has been used in the evaluation of 2 other therapeutic modalities (AZT combined with VGC and rituximab combined with liposomal doxorubicin) in the treatment of KSHV-MCD. These response criteria assess responses in several categories that are believed to be associated with disease activity, so as to record this in a unified way. We anticipate that the results obtained will continue to shed light on the benefits attained with therapy and also provide guidance to refine response criteria in subsequent trials in this disease.

Response criteria will be evaluated in each of three categories, clinical, biochemical, and radiographic. Clinical and biochemical responses will be evaluated at each visit, and radiographic responses will be evaluated for all scheduled CT scans. The baseline for response criteria evaluations will be based on the Day1 Cycle 1 evaluation for clinical and laboratory parameters for any given therapy, as well as baseline CT scans as defined in Section **3.4**. Any new clinical, laboratory, or radiologic abnormalities that are probably or definitely attributed to MCD and that occur between baseline and the end of the final cycle of a given therapy must also resolve in order to be considered a complete response. Only those abnormalities attributed as probably or definitely related to the disease will be included in the response assessment.

Given the waxing and waning course of MCD, patients may have progression in a given category at the time of interval evaluations. However, if on clinical evaluation there is evidence of clinical benefit, even though there may be lack of formal response in one or more of the categories, consideration will be given to continued study of that treatment.

Best response will be described for clinical, biochemical and radiographic categories for each subject. Additionally, best overall response and end of therapy overall response will be assessed for any given treatment based on all three response categories (clinical, biochemical, radiographic), as defined in Section **6.2.1.2.4**.

6.2.1.2 Definition of Responses (Also, See **Appendix 2**: KSHV-associated multicentric Castleman disease response criteria (Evaluated in 04-C-0275))

6.2.1.2.1 Clinical Response

6.2.1.2.1.1 **Complete Response (CR)** - full resolution of signs and symptoms attributable to MCD lasting at least 3-4 weeks (depending on therapeutic intervention) and normalization of all involved nodal areas.

6.2.1.2.1.2 **Symptom Free Disease (SFD)-** full resolution of symptoms that are attributable to MCD not yet lasting at least 3-4 weeks (depending on therapeutic intervention)

6.2.1.2.1.3 **Partial Response (PR)** – Symptoms attributed to MCD will be assigned a NCI CTC grade-equivalent. Improvement in at least 50% of the MCD-attributed signs and

symptoms by at least 1 NCI CTC grade-equivalent with remaining signs and symptoms showing no disease-related NCI CTC grade-equivalent increase lasting at least lasting at least 3-4 weeks (depending on therapeutic intervention) will be scored a partial clinical response

6.2.1.2.1.4 **Stable disease (SD)** – no change in signs and symptoms of MCD that meet criteria for PR, SFD, CR nor PD. The assignment of SD will be further annotated by the categories: improved, mixed response, stable, or worsened. These categories will be assigned based on changes from day 1 to the last day of any given cycle, as they are designed to describe short-term changes in clinical status. This subcategorization of SD will be used for exploratory analysis only, and will NOT be used in designating and reporting Best Response or End of Treatment Response.

Improved (I): improvement in one or more symptoms or signs by ≥ 1 grade with no new symptoms and no symptoms worsening by ≥ 1 grade.

Mixed Response (M): improvement in one or more symptoms or signs by ≥ 1 grade in combination with new symptoms or one or more symptoms worsen by ≥ 1 grade.

Stable (S): no change in grade of any symptom.

Worsened (W): No symptoms improve, and either new symptoms develop or one or more symptoms worsen by ≥ 1 grade.

6.2.1.2.1.5 **Progressive disease (PD)** – worsening of 2 or more signs and/or symptoms attributed to MCD by ≥ 1 grade

6.2.1.2.1.6 **Major Clinical Response =** Complete Response + Symptom Free Disease + Partial Response.

6.2.1.2.2 Biochemical Response

Five biochemical parameters are used in response assessment, hemoglobin, platelet count, C-reactive protein, sodium, and albumin.

6.2.1.2.2.1 **Complete response CR** - normalization of all biochemical tests related to MCD lasting at least 3-4 weeks (depending on therapeutic intervention).

6.2.1.2.2.2 **Partial PR** – 50% improvement in all biochemical tests related to MCD lasting at least 3-4 weeks (depending on therapeutic intervention).

6.2.1.2.2.3 **Stable disease SD** – no significant change in biochemical parameters related to MCD that meet criteria for PR, CR or PD. The assignment of SD will be further annotated by the categories: improved, mixed response, stable, or worsened. These categories will be assigned based on changes from day 1 to the last day of any given cycle, as they are designed to describe short-term changes in the laboratory parameters used in the evaluation of biochemical responses status. Direction of laboratory changes do not need to change the CTC grade, but must be larger than that the intra-assay variability of the test. The following minimum changes must be noted: Na - ≥ 2mmol/L, albumin ≥0.2 g/dL, platelets ≥ platelets/uL5 K/uL,

hemoglobin ≥ 0.2 g/dL, CRP ≥ 0.5 mg/L. This subcategorization of SD will be used for exploratory analysis only, and will NOT be used in designating and reporting Best Response or End of Treatment Response.

6.2.1.2.2.4 **Progressive disease (PD)** – 25% worsening of at least 2 biochemical parameters related to MCD by ≥ 1 grade, or clear deterioration of one parameter that has impact of physiologic or health status.

6.2.1.2.2.5 **Major Biochemical Response** = Complete Response + Partial Response.

6.2.1.2.3 Radiographic Response

The optimal imaging modality for monitoring MCD and evaluating radiographic response has not been defined. KSHV-MCD is a lymphoproliferative disease marked by multifocal lymphadenopathy. Diffuse splenomegaly is an additional common finding. Radiographic response criteria in this protocol have been modeled on the radiographic response criteria for malignant lymphoma studies as published by the International Working Group(67,68). Special attention has been given to splenic findings given the previously described natural history of MCD. CT scans will be used to define response criteria. PET scans will be explored as an imaging modality within the protocol, but are not used for defining radiographic response. As outlined in Revised Response Criteria published by the International Working Group, lymph node evaluation will based on serial evaluation of up to SIX dominant pathologic lymph nodes. Any lymph node >1.5 cm at follow-up evaluation will also be evaluated if a subject otherwise potentially meets criteria for CR or CRu radiographic response, even if it was not identified as one of the original six dominant pathologic lymph nodes.

Given the prominence of splenomegaly, a separate response will be noted for lymph nodes and for the spleen. Unlike the International Working Group lymphoma criteria, which generally focus on nodules within the spleen, MCD response criteria in this protocol will focus on overall spleen size. In order to obtain an overall complete radiographic response, both spleen and lymph nodes must meet criteria as defined below. As an additional modification, pleural effusions must resolve in order to obtain a complete response. Although unconfirmed complete response (CRu) has been removed from the 2007 revision of radiographic response criteria for malignant lymphoma, we have maintained a modified version of CRu that is potentially clinically meaningful in MCD.

The following responses are based on CT scan of neck, chest, abdomen and pelvis to evaluate sites of disease.

6.2.1.2.3.1 **Complete response (CR) -** All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy) for at least one month. For the SIX target lesions, involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to ≤ 1 cm in their greatest transverse after treatment or by more than 75% in the sum of the products of the greatest diameters (SPD). The spleen, if enlarged (>12 cm in the longest dimension) before therapy on the basis of a CT scan

must have regressed <12 cm and must not be palpable on physical examination. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present. All pleural effusions must have resolved.

6.2.1.2.3.2 **Complete response unconfirmed (CRu) -** Any residual lymph node mass > 1.5 cm in greatest transverse diameter that has regressed by > 75% in sum of the products of the greatest diameters and does not change over one year of observation and/or residual splenomegaly >12 cm in longest dimension, but that has regressed by 75% and does not change over one year. In organs involved by disease, any residual lesions that have decreased by > 75% in sum of the products of the greatest diameters or are < 1 cm, are consistent with scar, and stable over the last two treatments will be considered to fulfill criteria for CR.

6.2.1.2.3.3 **Partial response (PR)** - For lymph nodes: 50% or greater decrease in the sum of the products of the longest perpendicular diameters (SPD) of the six dominant lymph nodes lasting for a period of at least one month. No increase in size of other nodes, For spleen: a 50% decrease in longest dimension of spleen [decrease > (baseline length – 12 cm) / 2] No new lesions, including pleural effusions, may appear.

6.2.1.2.3.4 **Stable disease (SD)** - tumor measurements not meeting the criteria of CR, PR, or PD.

6.2.1.2.3.5 **Progressive disease (PD)** - For lymph nodes, an increase of 25% or more in the sum of the products of the longest perpendicular diameters of all measured lesions compared to the smallest previous measurements during a given therapy. For spleen, an increase of greater than 25% in the longest dimension of the spleen compared to the smallest previous measurement during a given therapy., or the appearance of any new lesion(s), including pleural effusions, will be considered progressive disease.

6.2.1.2.3.6 **Major Radiographic Response** = Complete Response + Complete Response unconfirmed + Partial Response.

6.2.1.2.4 Overall Response

Overall response will be determined based on responses in clinical, biochemical, and radiographic response criteria. Overall responses are defined as the highest response below.

6.2.1.2.4.1 **Complete response (CR)** = Complete response in all categories

6.2.1.2.4.2 **Partial response (PR)** = Partial response or better in all categories

6.2.1.2.4.3 **Stable disease (SD)** = Stable disease or better in all categories

6.2.1.2.4.4 **Progressive disease (PD)** = Progressive disease in any one category

6.2.2 KSHV-MCD Clinical Benefit Response Criteria

6.2.2.1 Background

In the literature, there are no established criteria for responses in MCD (including KSHV-MCD) and no uniformity among various articles. Some studies report results in only in general terms of resolution of symptoms and fever(14) or a composite of symptoms, fever, and an elevated C-reactive protein.(13) We developed and have tested the use of a set of NCI KSHV-MCD response criteria in our current ongoing studies (especially 04-C-0275), which are more rigorous, and included more biochemical and radiographic endpoints. Within 04-C-0275, we have gained experience with these criteria with two treatment modalities: AZT/VGC and rituximab combined with liposomal doxorubicin. While we found these criteria to be useful and to provide a complete picture, they have the disadvantage of being rather cumbersome. Also, the radiographic responses usually lagged behind the other responses. Based on experience within a total of 28 patients in this Natural History protocol, our sense was that the evaluation of therapy would benefit from a combined primary response criteria that included the most important clinical (symptomatic) criteria and the most important laboratory criteria. From discussions with other investigators working on MCD in the United States and Europe, it appears that these other groups are moving in a similar direction.

With this experience, we have developed **KSHV-MCD Clinical Benefit Response Criteria** as a more streamlined tool for assessment of patients receiving therapy for KSHV-MCD. These criteria do not depend on radiographic findings. As such, it allows for same-day assessment of clinical benefit utilizing common symptoms and clinical laboratories. To evaluate clinical benefit in patients with KSHV-MCD receiving therapy, clinical and laboratory responses are assessed in aggregate, and compared to baseline. These criteria allow for assessment of patients who may be deriving meaningful clinical benefit from therapy, but have not necessarily achieved an overall complete response by the original NCI KSHV-MCD response criteria. In an effort to harmonize response criteria across researchers in the field, we hope to propose these criteria for use across KSHV-MCD studies, and will compare it our prior methodology (see Section **8.3.2.2**). We plan to simultaneously assess the patients using the original NCI KSHV-MCD Response Criteria to gather information on the two criteria. Ultimately, our sense is that responses in the disease will involve tracking Clinical Benefit response Criteria and Radiographic responses separately.

In brief, assessment of **KSHV-MCD Clinical Benefit Response** is made on evaluation of eight *indicator abnormalities* (four symptom groups and four laboratory parameters) that are most closely associated with disease activity.

6.2.2.2 KSHV-MCD Clinical Benefit Response Criteria

Indicator Abnormalities Assessed in Responses	
Symptoms	*Laboratory Abnormalities*
Fever (includes chills and rigors)	Elevated C-reactive protein
Fatigue (includes lethargy)	Thrombocytopenia
Gastrointestinal (includes nausea and anorexia)	Anemia
Respiratory (includes airway hyperreactivity and cough)	Hypoalbuminemia

For the purposes of response assessment, clinical symptoms attributed to MCD will be assigned an NCI-CTCAE grade equivalent, with response assessment based on changes in grade severity or symptom resolution.

Increases in hemoglobin in patients who have received a transfusion do not count towards PR or CR for 3 weeks, and increases in albumin or platelet count in patients who have received a transfusion do not count towards PR or CR for 7 days.

6.2.2.3 Complete Response (CR):

Full resolution of all clinical symptoms and laboratory abnormalities (whether or not these are indicator abnormalities) probably or definitely attributable to MCD, lasting at least 3 weeks.

6.2.2.4 Partial Response (PR):

PR is assessed based on the eight indicator abnormalities above. At least 50% of the abnormalities probably or definitely attributed to KSHV-MCD must improve by the minimum amounts specified below to attain PR.

Only abnormalities present in a specific patient at baseline may count toward the achievement of a PR (e.g. if six of indicator abnormalities are present at baseline, at least three must meet the specified criteria to be considered a PR).

Improvement in symptoms require at least 1 CTCAE grade equivalent improvement. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KSHV-MCD must improve by at least 1 CTCAE grade equivalent to consider the group as a whole to be improved.

Improvement in for each laboratory parameters requires either normalization of the lab value or else the following:

6.2.2.4.1 C-reactive protein reduction to ≤50% of baseline

6.2.2.4.2 Hemoglobin increment 2g/dL not explained by transfusion

6.2.2.4.3 Platelet increment ≥50 K/uL not explained by transfusion

6.2.2.4.4 Albumin increment ≥1g/dL not explained by transfusion

To be considered a partial response, there may be no new indicator abnormalities probably or definitely attributed to KSHV-MCD; no indicator symptom may worsen by ≥1 CTCAE grade equivalent; and no indictor laboratory abnormality may worsen by the amount given in the criteria for progressive disease.

6.2.2.5 **Stable disease (SD):** no change in signs and symptoms of KSHV-MCD that meet criteria for any of CR, PR or PD.

6.2.2.6 Progressive disease (PD):

PD is assessed based on the eight indicator abnormalities above. At least two indicator abnormalities must deteriorate by the minimum amounts specified below to constitute PD. The development of new indicator abnormalities not present in a specific patient at baseline is incorporated in the assessment of PD

Deterioration in signs and symptoms require at least 1 CTCAE grade equivalent increase in severity. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms

within the group are present at least half of those attributable to KSHV-MCD must increase in severity by at least 1 CTCAE grade equivalent to consider the group as a whole to have deteriorated.

Deterioration for each laboratory parameter requires an abnormal laboratory value meeting the following criteria:

6.2.2.6.1 C-reactive protein increase by ≥50% of baseline (or the upper limit of normal, whichever is greater)

6.2.2.6.2 Hemoglobin decrement 2g/dL not otherwise explained

6.2.2.6.3 Platelet decrement ≥25 K/uL not otherwise explained

6.2.2.6.4 Albumin decrement ≥0.5g/dL not otherwise explained

6.2.3 Kaposi's Sarcoma Response

6.2.3.1 Background

Evaluation of the response of KS to an agent or regimen is difficult to grade by means of commonly used oncologic definitions. However, in an effort to standardize the evaluation of therapy against KS, the AIDS Clinical Trial Group Oncology Committee has devised a set of staging and response definitions for KS. We will use a modification of these criteria to assess responses, which is consistent with the criteria used in our previous KS studies. It should be noted that there is some observer variability in the evaluation of the number, size, nodularity, and color of lesions, and this must be taken into account when measurements are interpreted. (See **Appendix 4**)

For evaluation of less than complete responses in patients with more than 50 lesions at entry, only the previously selected 1 - 3 representative areas that contain at least 20 lesions will be considered. However, complete responses still require the absence of any detectable disease over the entire body (i.e. not confined to the representative areas).

6.2.3.2 Evaluation Schedule

For patients with Kaposi's sarcoma (KS), clinical evaluation of the response of their KS will be assessed at baseline, week 7, and at off-study visit. (See Study Calendar, Section **3.4**) When assessed, KS responses will be reported separately from responses assessed for MCD, and will not be considered part of the Overall Response of MCD outlined in Section **6.2.1.2**. Additional studies at the end of therapy may be required for patients with visceral KS, these include, but are not limited to: bronchoscopy, gastrointestinal endoscopy, chest X-ray, and CT-scans.

6.2.3.3 Definition of Kaposi Sarcoma Responses

6.2.3.3.1 **Complete Response**

6.2.3.3.1.1 The absence of any detectable residual disease, including tumor associated edema, persisting for at least 4 weeks.

6.2.3.3.1.2 For patients with pigmented macular skin lesions persisting after apparent complete response, a biopsy of at least one representative lesion is required to document the absence of malignant cells.

6.2.3.3.1.3 For patients with visceral disease, an attempt at restaging with appropriate endoscopic or radiographic procedures should be made. If such procedures are medically contraindicated, the patient may be classified as having a clinical CR

6.2.3.3.2 **Clinical Complete Response**

6.2.3.3.2.1 The absence of any detectable residual disease, including tumor associated edema, persisting for at least 4 weeks.

6.2.3.3.2.2 For patients with pigmented macular skin lesions persisting after apparent complete response, if a representative lesion has not been biopsied.

6.2.3.3.2.3 For patients with visceral disease, the diagnostic radiologic or endoscopic study should be repeated if not medically contraindicated and found to be negative for evidence of disease. If such procedures are medically contraindicated but the patient has no clinical evidence of visceral disease, the patient may be classified as having a clinical CR

6.2.3.3.3 **Partial Response**

6.2.3.3.3.1 No progressive disease (see below and noting, that single lesions which split up into 2 or more smaller lesions during the course of treatment will still be counted as one); no new lesions occurring in previously uninvolved areas of the body; no new visceral sites of involvement or the appearance or worsening of tumor-associated edema or effusions and:

6.2.3.3.3.2 A 50% or greater decrease in the number of previously existing lesions lasting for at least 4 weeks or

6.2.3.3.3.3 A 50% or greater decrease in the sum of the size (sum of the products of the largest perpendicular diameters) of the 5 marker lesions lasting for at least 4 weeks.

6.2.3.3.3.4 Complete flattening of at least 50% of all previously raised lesions (i.e., 50% of all previously nodular or plaque-like lesions become macular) lasting for at least 4 weeks or

6.2.3.3.3.5 A 50% decrease in radiologically measurable visceral lesions sustained without evidence of re-growth for at least 4 weeks or

6.2.3.3.3.6 Patients who otherwise meet the criteria for a CR but still have residual tumor-associated edema or effusions will be classified as having a PR.

6.2.3.3.4 **Progressive Disease**

6.2.3.3.4.1 For those criteria that involve measurement of lesions in the clinic, the designation of progression should be made, when feasible, only when the criteria below have been

met in two measurements spaced at least 1 week apart. For the assignment of progressive disease for the primary outcome analysis, progression will be defined in comparison to baseline measurements.

6.2.3.3.4.2 An increase of 25% or more over baseline in the number of lesions and/or the size (sum of the products of the largest perpendicular diameters) of the marker lesions or

6.2.3.3.4.3 A change in character from macular to plaque-like or nodular of at least 25% of the lesions or

6.2.3.3.4.4 New visceral sites of involvement or progression of visceral disease or

6.2.3.3.4.5 The development of new or increasing tumor-associated edema or effusion that lasts at least 1 week and interferes with the patient's normal activities.

6.2.3.3.5 **Stable Disease**

Any tumor measurement not meeting the criteria for Complete Response, Partial Response, or Progressive Disease.

6.2.3.3.6 **Overall Response (Major KS Response)**

Overall Response = Complete Response + Clinical Complete Response + Partial Response.

6.2.3.4 Progression-free survival

As Kaposi's sarcoma is a disease in which recurrence is common, progression will be determined based on progression from best response. Progression must be documented on two visits at least 1 week apart. Progression-free survival is defined as the time interval from the date of enrollment to the date of progression from best response. The criteria for progression from best response include:

6.2.3.4.1 An increase of 25% or more over best response in the number of lesions and/or the size (sum of the products of the largest perpendicular diameters) of the marker lesions or

6.2.3.4.2 A change in character from macular to plaque-like or nodular of at least 25% of the lesions or

6.2.3.4.3 New visceral sites of involvement or progression of visceral disease or

6.2.3.4.4 The development of new or increasing tumor-associated edema or effusion that lasts at least 1 week and interferes with the normal activities

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40). All

appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

7 NIH REPORTING REQUIREMENTS / DATA SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING -

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here.

If the study is under review by an IC specific IRB, the following should be noted:

- Reports referenced in policy 801 are made to the IC Specific IRB rather than to the OHSRP Office of Compliance and Training.
- Section 5.2 of the policy 801 does not apply
- Policy 802 does not apply

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.2.3 NCI Clinical Director Reporting

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at dahutw@mail.nih.gov and to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.3 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.3.1 Principal Investigator/Research Team

The clinical research team will meet regularly (weekly when feasible) to discuss patients on this study receiving treatment that week. Decisions related to study treatment will be discussed and information from prior patients treated on the study will be used to guide any issues related to management of toxicities seen on the study, where appropriate.

Patient data will be collected in a timely manner and reviewed by a physician Associate Investigator and/or the Principal Investigator, Robert Yarchoan, for toxicity. Any toxicity ≥ Grade 3 will be reviewed by the Principal Investigator or physician Associate Investigator.

Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The Principal Investigator will do continuous, close monitoring of this pilot study, and a Data and Safety Monitoring Board will not be used. CCR Safety Monitoring Committee (SMC) review is not necessary for this study. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

8.1 RACIAL/GENDER MAKE-UP

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section **2.1**. Outreach efforts will be made to extend accrual to a representative population. As a practical matter, it should be noted that historically in the US, KSHV associated malignancies have been relatively rare in females with HIV infection. However, our more recent studies have increasingly accrued female immigrants from Africa.

8.2 AGE EXCLUSION

Patients under the age of 18 will be excluded. This is a pilot clinical study of an agent with unknown safety profile in children and adolescents in a disease that is rare in children. Tocilizumab is current FDA approved for treatment of adults with rheumatoid arthritis.

8.3 SAMPLE SIZE CONSIDERATIONS AND STATISTICAL PLAN

8.3.1 Primary Objective

The primary objective of this study is to estimate clinical benefit of tocilizumab 8mg/kg every 2 weeks for up to 12 weeks in patients with symptomatic KSHV-MCD using a modified KSHV-MCD Clinical Benefit Response Criteria.

Sample size was determined based on the primary objective. Clinical evidence of tocilizumab benefit will be based on achieving a Partial Response or better using the KSHV-MCD Clinical Benefit Response Criteria (**Appendix 1**)

The study will be conducted using a two-stage phase II optimal design with alpha=0.10 and beta=0.10, in order to rule out 20% KSHV-MCD Clinical Benefit Partial Response or better as the best response and targeting a 50% KSHV-MCD Clinical Benefit Partial Response or better as a best response. Using this design, the study will initially accrue 10 patients in the first stage. If 0-2 of 10 have a Clinical Benefit Partial Response or better, then accrual would stop, while 3+/10 with responses would allow accrual to 17 total. If 3-5 of 17 total patients respond, this would be inadequate, while 6+/17 with a response would be consistent with 50% and merit further evaluation in a subsequent study. The probability of early termination under the null hypothesis (20%) is 68%.

8.3.2 Secondary Objectives

The following secondary outcomes will be evaluated with the following statistical considerations.

8.3.2.1 Estimate best clinical, biochemical, radiographic, and overall responses in patients with

KSHV-MCD treated for up to 12 weeks with tocilizumab 8mg/kg every 2 weeks

Biochemical, radiographic and overall responses, will also be described using NCI KSHV-MCD Response criteria, as we have done in other studies of KSHV-MCD (See Section **1.2**, **Table 1**). Best responses in each category will be tabulated, with major responses are defined as follows:

Major Clinical Response = Complete Response + Symptom Free Disease + Partial Response
Major Biochemical Response = Complete Response + Partial Response.
Major Radiographic Response = Complete Response + Complete Response unconfirmed + Partial Response.

8.3.2.2 Evaluate responses as graded by the KSHV-MCD Clinical Benefit Response Criteria compared to the 04-C-0275 NCI KSHV-MCD response criteria

Best responses in each category will be tabulated using NCI-KSHV-MCD response criteria and KSHV-MCD Clinical Benefit Criteria as outlined above.

Additionally, at each assessment time-point, KSHV-MCD Clinical Benefit Response will be compared to:

8.3.2.2.1 The combination of Clinical Response and Biochemical Response Criteria, using the 04-C-0275 NCI KSHV-MCD criteria using the following categorization:

8.3.2.2.1.1 **Complete response (CR)** = Complete response in both categories

8.3.2.2.1.2 **Partial response (PR)** = Partial response or better in both categories

8.3.2.2.1.3 **Stable disease (SD)** = Stable disease or better in both categories

8.3.2.2.1.4 **Progressive disease (PD)** = Progressive disease in any one category

8.3.2.2.2 Clinical responses alone using NCI KSHV-MCD criteria, using the following categorization:

8.3.2.2.2.1 **Complete response (CR)** = Complete response + symptom free disease

8.3.2.2.2.2 **Partial response (PR)** = Partial response

8.3.2.2.2.3 **Stable disease (SD)** = Stable disease

8.3.2.2.2.4 Progressive disease (PD) = Progressive disease

A kappa statistic as well as other general measures of association and descriptive statistics may be calculated for each of these two comparisons.

8.3.2.3 Evaluate of safety and tolerability of tocilizumab alone and combined with AZT/VGC

Toxicities (including those possibly, probably, and definitely attributed to study treatment) will be assessed by grade. Toxicities will be evaluated both per cycle and per patient. All patients will be considered evaluable for toxicity.

8.3.2.4 In patients with inadequate response to tocilizumab monotherapy: explore preliminarily the activity of tocilizumab 8mg/kg every 2 weeks, combined with AZT 600 mg orally q6 hours and VGC 900 mg orally q12 hours on days 1-5 of a 14-day cycle.

We will gather preliminary data on the activity of tocilizumab combined with AZT and VGC in evaluable patients. All responses will be based on KSHV-MCD Clinical Benefit Response Criteria. The number of patients receiving this combination will be determined by the number with an indication for treatment escalation with the addition of AZT and VGC. We will also continue to assess the NCI KSHV-MCD (04-C-0275) response criteria.

8.3.2.5 Evaluate effect of tocilizumab on KS

In patients with concurrent Kaposi sarcoma, we will describe best KS response, using modified ACTG Response criteria, outlined in Section **6.2.3**.

8.3.2.6 Evaluate progression-free and overall survival

For survival endpoints, subjects will be followed for up to approximately 4 months on this protocol. Long-term follow up will be feasible in patients co-enrolled in the KSHV-MCD Natural History Protocol, 04-C-0275 (See Sections **2.3** and **6.1.3**.). Progression-free and overall survival will be described using Kaplan-Meier methodology.

8.3.2.7 Evaluate effect of tocilizumab on biochemical, immunologic, and virologic correlates of symptomatic KSHV-MCD and IL-6 excess

We will evaluate longitudinal effect of tocilizumab monotherapy on individual biochemical (c-reactive protein, hemoglobin, platelets, albumin, sodium, and ferritin), virologic (HHV-8 viral load, vIL-6), and immunologic (quantitative immunoglobulins, free light chains, sIL-6 receptor, IL-6, IL-10, and other inflammatory cytokines, anti-k 8.1 and anti-LANA antibodies) parameters, using both descriptive analyses and non-parametric comparisons at relevant time points.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

This protocol is designed for the treatment of all adult subjects with symptomatic KSHV-associated MCD. The main epidemiologic group that would be potential subjects is those with HIV. KSHV-MCD also occurs in elderly and other immunosuppressed populations. Subjects are generally from areas where the prevalence of KSHV infection is high. In the US, KSHV infection is most common in men who have sex with men (MSM). At the HAMB, women who have participated in our ongoing KSHV-MCD studies are immigrants from Africa.

Strategies for recruitment will include announcements on ClinicalTrials.gov, letters to referring physicians, targeting HIV providers and those who provide primary care to the African immigrant community, AIDS treatment bulletins, and the International Castleman's Disease Organization, and through a Google campaign.

9.2 PARTICIPATION OF CHILDREN

Patients under the age of 18 will be excluded. This is a pilot clinical study of an agent with unknown safety profile in children and adolescents in a disease that is rare in children. Tocilizumab is currently FDA approved for treatment of adults with rheumatoid arthritis.

9.3 PARTICIPATION OF SUBJECTS UNABLE TO CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **9.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

9.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the patient or the patients surrogate, and a signed informed consent document will be obtained.

9.4.1 Potential Benefits

The potential benefit to individual patient-volunteers is that the protocol therapy may result in improvement of symptomatic KSHV-MCD associated symptoms. It may also contribute towards the long-term control of KSHV-MCD, without leading to KS flares, as has been described in patients receiving B-cell depleting therapy with rituximab. Participation in a clinical study will help researchers better understand the pathophysiology of KSHV-MCD and KS, as well as allow for an evaluation of tocilizumab in patients with symptomatic KSHV-MCD.

9.4.2 Potential Risks and Discomforts

There may be prospect of direct benefit to the patient-volunteers on this study. Potential risks include the potential toxicity of tocilizumab in this patient population. Additionally, the safety of tocilizumab has not evaluated when used in combination with AZT and VGC. See Section **10** for potential toxicities of individual agents. Patients will be followed on study until resolution of acute adverse events, and clinical investigators will ensure that subjects receive appropriate medical care for any adverse events. Measures to prevent infectious complications are outlined in Section **4.6**. Participation in clinical study may also have discomforts and inconveniences associated with additional studies. Additional risks include the small risk of complications

related to biopsies (when required) and blood draws, which include pain, bleeding, seroma formation, and infection. The PI of the study will monitor all adverse events, following the Data and Safety Monitoring Plan outlined in Section **7.3**.

9.4.3 Alternatives

Alternative options for patients with symptomatic KSHV-MCD include treatment with other therapeutic regimens evaluated in 04-C-0275, or treatment off-study using one or more agents with activity in KSHV-MCD, such as rituximab. Observation is usually discouraged in patients with symptomatic disease.

9.5 Risks/Benefits Analysis

The risks to individual study subjects are reasonable in relation to the anticipated benefits. This protocol evaluates tocilizumab, which is active in multicentric Castleman disease in patients not infected with KSHV, and is approved for this indication in Japan. Its use in KSHV-MCD has strong clinical and preclinical rationale and an acceptable infectious complication profile in comparison to rituximab. Potential therapeutic benefits include limitation of cumulative anthracycline dosing (04-C-0275 has explored rituximab in combination with liposomal doxorubicin for this patient population), as well as a favorable toxicity profile in relation to worsening of concurrent KS. Additional risks include potential additive toxicities in patients requiring treatment intensification with AZT/VGC, as well as discomforts and inconvenience of additional studies associated with participation in a clinical study. There is no FDA approved therapy for KSHV-MCD, and there is no consensus on the standard of care in this disease. The major alternative for patients with symptomatic KSHV-MCD is participation in another study, or treatment off-study with a regimen with described activity. The comparative risks and benefits are acceptable for an early phase clinical study, when compared to the alternatives for patients with symptomatic KSHV-MCD.

9.6 Consent and Assent Process and Documentation

Informed written consent will be obtained in all patients on this trial. There will be no minors enrolled < 18 years old, so assent is unnecessary. All potential participants will be provided an IRB-approved informed consent form to review prior to their screening visit. The Principal Investigator or a physician Associate Investigator will discuss the risks, benefits and alternatives to participation in this study, and answer all patient questions regarding study participation. Informed consent will be obtained on an IRB approved informed consent form, with signatures obtained from the consenting physician, a witness, and the study subject. The original informed consent will be stored in the patient's official hospital records, with a copy stored in the patient's research chart, and a copy provided to the study subject.

9.6.1 Telephone Re-Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented in the medical record.

10 PHARMACEUTICAL INFORMATION

10.1 TOCILIZUMAB

Please, refer to package insert.

10.1.1 Source:

Tocilizumab (TCZ) is commercially available under the trade name Actemra®, and will be obtained from commercial sources from the Clinical Center Pharmacy.

10.1.2 Administration procedures:

10.1.2.1 Allow the fully diluted ACTEMRA solution to reach room temperature prior to infusion.

10.1.2.2 The infusion should be administered over 60 minutes, and must be administered with an infusion set. Do not administer as an intravenous push or bolus.

10.2 ZIDOVUDINE (AZT)

10.2.1 Please, refer to package insert.Source:

AZT is commercially available for both oral and IV injection use, and will be obtained by the Clinical Center pharmacy from commercial sources. AZT oral formulations include Retrovir® tablets containing 300 mg. Retrovir® intravenous infusion is formulated in a preservative free sterile water for injection with 10 mg AZT in each milliliter. The maximum dose of 2.4 gram AZT daily in this protocol is higher than the recommended maximum dose in standard practice for HIV disease, but substantially lower than the 50-gram doses associated with acute overdose complications. Additionally, the NCI phase I trial treated patients at up to 45 mg/kg/day intravenously, (approximately 3.1 grams daily) for two weeks, followed by up to 90 mg/kg/day orally (approximately 6.2 grams daily) for four weeks(69). Toxicities were similar as that seen at lower doses and were primarily myelosuppression, tremors and confusion. Thus, doses for this trial are conservative in magnitude and cycle duration (generally less than 2 weeks dosing at any given time).

10.2.2 Administration procedures

10.2.2.1 Intravenous AZT

Infuse intravenously over 1 hour. Intravenous AZT should not be used if the patient is able to take oral AZT.

10.3 VALGANCICLOVIR / GANCICLOVIR

10.3.1 Please, refer to package insert.Source

Commercially available for both oral (VGC) and IV injection use (ganciclovir), and will be obtained by the Clinical Center pharmacy from commercial sources.

10.3.2 Administration procedures:

10.3.2.1 Intravenous administration: infuse 5 mg/kg over 1 hour every 12 hours in patients with normal renal function (refer to section 3.3.2.6.3. for instructions for use in renally impaired individuals).

10.3.2.2 Oral administration: two 450 mg VGC (Valcyte®) tablets with food orally, every 12 hours.

10.3.2.3 DO NOT SUBSTITUTE ORAL CYTOVENE CAPSULES

11. REFERENCES

1. Uldrick, T. S., Polizzotto, M., O'Mahony, D., Aleman, K., Wyvill, K. M., Steinberg, S. M., Pittaluga, S., Marshall, V., Whitby, D., Tosato, G., Little, R. F., and Yarchoan, R. (2010) Rituximab Combined with Liposomal Doxorubicin (R-Dox) in HIV-Infected Patients with Severe Kaposi Sarcoma-associated Herpes Virus (KSHV) – associated Multicentric Castleman Disease (MCD). . in *12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies: Basic, Epidemiologic, and Clinical Research.* , Bethesda, MD.

2. Brandt, S. J., Bodine, D. M., Dunbar, C. E., and Nienhuis, A. W. (1990) Dysregulated interleukin 6 expression produces a syndrome resembling Castleman's disease in mice. *J Clin Invest* **86**, 592-599

3. Uldrick, T. S., Wang, V., O'Mahony, D., Aleman, K., Wyvill, K. M., Marshall, V., Steinberg, S. M., Pittaluga, S., Maric, I., Whitby, D., Tosato, G., Little, R. F., and Yarchoan, R. (2010) An Interleukin-6-Related Systemic Inflammatory Syndrome in Patients Co-Infected with Kaposi Sarcoma-Associated Herpesvirus and HIV but without Multicentric Castleman Disease. *Clin Infect Dis* **51**, 350-358

4. Oksenhendler, E., Carcelain, G., Aoki, Y., Boulanger, E., Maillard, A., Clauvel, J. P., and Agbalika, F. (2000) High levels of human herpesvirus 8 viral load, human interleukin-6, interleukin-10, and C reactive protein correlate with exacerbation of multicentric castleman disease in HIV-infected patients. *Blood* **96**, 2069-2073.

5. Aoki, Y., Jaffe, E. S., Chang, Y., Jones, K., Teruya-Feldstein, J., Moore, P. S., and Tosato, G. (1999) Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. *Blood* **93**, 4034-4043

6. Neipel, F., Albrecht, J. C., Ensser, A., Huang, Y. Q., Li, J. J., Friedman-Kien, A. E., and Fleckenstein, B. (1997) Human herpesvirus 8 encodes a homolog of interleukin-6. *J Virol* **71**, 839-842

7. Nicholas, J., Ruvolo, V. R., Burns, W. H., Sandford, G., Wan, X., Ciufo, D., Hendrickson, S. B., Guo, H. G., Hayward, G. S., and Reitz, M. S. (1997) Kaposi's sarcoma-associated human herpesvirus-8 encodes homologues of macrophage inflammatory protein-1 and interleukin-6. *Nat Med* **3**, 287-292

8. Aoki, Y., Tosato, G., Fonville, T. W., and Pittaluga, S. (2001) Serum viral interleukin-6 in AIDS-related multicentric Castleman disease. *Blood* **97**, 2526-2527

9. Nishimoto, N., Kanakura, Y., Aozasa, K., Johkoh, T., Nakamura, M., Nakano, S., Nakano, N., Ikeda, Y., Sasaki, T., Nishioka, K., Hara, M., Taguchi, H., Kimura, Y., Kato, Y., Asaoku, H., Kumagai, S., Kodama, F., Nakahara, H., Hagihara, K., Yoshizaki, K., and Kishimoto, T. (2005) Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman's disease. *Blood*, 2004-2012-4602

10. Davis, D. A., Singer, K. E., Reynolds, I. P., Haque, M., and Yarchoan, R. (2007) Hypoxia enhances the phosphorylation and cytotoxicity of ganciclovir and zidovudine in Kaposi's sarcoma-associated herpesvirus infected cells. *Cancer Res* **67**, 7003-7010

11. Uldrick, T. S., Polizzotto, M. N., Aleman, K., O'Mahony, D., Wyvill, K. M., Wang, V., Marshall, V., Pittaluga, S., Steinberg, S. M., Tosato, G., Whitby, D., Little, R. F., and Yarchoan, R. (2011) High-dose zidovudine plus valganciclovir for Kaposi sarcoma herpesvirus-associated multicentric Castleman disease: a pilot study of virus-activated cytotoxic therapy. *Blood*

12. Soulier, J., Grollet, L., Oksenhendler, E., Cacoub, P., Cazals-Hatem, D., Babinet, P., d'Agay, M. F., Clauvel, J. P., Raphael, M., Degos, L., and et al. (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* **86**, 1276-1280

13. Gerard, L., Berezne, A., Galicier, L., Meignin, V., Obadia, M., De Castro, N., Jacomet, C., Verdon, R., Madelaine-Chambrin, I., Boulanger, E., Chevret, S., Agbalika, F., and Oksenhendler, E. (2007) Prospective study of rituximab in chemotherapy-dependent human immunodeficiency virus associated multicentric Castleman's disease: ANRS 117 CastlemaB Trial. *J Clin Oncol* **25**, 3350-3356

14. Bower, M., Powles, T., Williams, S., Davis, T. N., Atkins, M., Montoto, S., Orkin, C., Webb, A., Fisher, M., Nelson, M., Gazzard, B., Stebbing, J., and Kelleher, P. (2007) Brief communication: rituximab in HIV-associated multicentric Castleman disease. *Ann Intern Med* **147**, 836-839

15. Shortt, J., and Spencer, A. (2006) Adjuvant rituximab causes prolonged hypogammaglobulinaemia following autologous stem cell transplant for non-Hodgkin's lymphoma. *Bone Marrow Transplant* **38**, 433-436

16. Stebbing, J., Gazzard, B., Newsom-Davis, T., Nelson, M., Patterson, S., Gotch, F., Mandalia, S., and Bower, M. (2004) Nadir B cell counts are significantly correlated with the risk of Kaposi's sarcoma. *Int J Cancer* **108**, 473-474

17. Huang, K. H., Bonsall, D., Katzourakis, A., Thomson, E. C., Fidler, S. J., Main, J., Muir, D., Weber, J. N., Frater, A. J., Phillips, R. E., Pybus, O. G., Goulder, P. J., McClure, M. O., Cooke, G. S., and Klenerman, P. (2010) B-cell depletion reveals a role for antibodies in the control of chronic HIV-1 infection. *Nat Commun* **1**, 102

18. Kimball, L. E., Casper, C., Koelle, D. M., Morrow, R., Corey, L., and Vieira, J. (2004) Reduced levels of neutralizing antibodies to Kaposi sarcoma-associated herpesvirus in persons with a history of Kaposi sarcoma. *J Infect Dis* **189**, 2016-2022

19. Burbelo, P. D., Issa, A. T., Ching, K. H., Wyvill, K. M., Little, R. F., Iadarola, M. J., Kovacs, J. A., and Yarchoan, R. (2010) Distinct profiles of antibodies to Kaposi sarcoma-associated herpesvirus antigens in patients with Kaposi sarcoma, multicentric Castleman disease, and primary effusion lymphoma. *J Infect Dis* **201**, 1919-1922

20. Kaplan, L. D., Lee, J. Y., Ambinder, R. F., Sparano, J. A., Cesarman, E., Chadburn, A., Levine, A. M., and Scadden, D. T. (2005) Rituximab does not improve clinical outcome in a randomized phase III trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin's lymphoma: AIDS-malignancies consortium trial 010. *Blood*

21. Carson, K. R., Evens, A. M., Richey, E. A., Habermann, T. M., Focosi, D., Seymour, J. F., Laubach, J., Bawn, S. D., Gordon, L. I., Winter, J. N., Furman, R. R., Vose, J. M.,

Zelenetz, A. D., Mamtani, R., Raisch, D. W., Dorshimer, G. W., Rosen, S. T., Muro, K., Gottardi-Littell, N. R., Talley, R. L., Sartor, O., Green, D., Major, E. O., and Bennett, C. L. (2009) Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the Research on Adverse Drug Events and Reports project. *Blood* **113**, 4834-4840

22. Staskus, K. A., Sun, R., Miller, G., Racz, P., Jaslowski, A., Metroka, C., Brett-Smith, H., and Haase, A. T. (1999) Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *J Virol* **73**, 4181-4187

23. Du, M. Q., Liu, H., Diss, T. C., Ye, H., Hamoudi, R. A., Dupin, N., Meignin, V., Oksenhendler, E., Boshoff, C., and Isaacson, P. G. (2001) Kaposi sarcoma-associated herpesvirus infects monotypic (IgM lambda) but polyclonal naive B cells in Castleman disease and associated lymphoproliferative disorders. *Blood* **97**, 2130-2136.

24. Chadburn, A., Hyjek, E. M., Tam, W., Liu, Y., Rengifo, T., Cesarman, E., and Knowles, D. M. (2008) Immunophenotypic analysis of the Kaposi sarcoma herpesvirus (KSHV; HHV-8)-infected B cells in HIV+ multicentric Castleman disease (MCD). *Histopathology* **53**, 513-524

25. Naresh, K. N., Trivedi, P., Horncastle, D., and Bower, M. (2009) CD20 expression in the HHV-8-infected lymphoid cells in multicentric Castleman disease. *Histopathology* **55**, 358-359

26. Dupin, N., Diss, T. L., Kellam, P., Tulliez, M., Du, M. Q., Sicard, D., Weiss, R. A., Isaacson, P. G., and Boshoff, C. (2000) HHV-8 is associated with a plasmablastic variant of Castleman disease that is linked to HHV-8-positive plasmablastic lymphoma. *Blood* **95**, 1406-1412.

27. Tosato, G., Seamon, K. B., Goldman, N. D., Sehgal, P. B., May, L. T., Washington, G. C., Jones, K. D., and Pike, S. E. (1988) Monocyte-derived human B-cell growth factor identified as interferon-beta 2 (BSF-2, IL-6). *Science* **239**, 502-504

28. Tosato, G., Tanner, J., Jones, K. D., Revel, M., and Pike, S. E. (1990) Identification of interleukin-6 as an autocrine growth factor for Epstein-Barr virus-immortalized B cells. *J Virol* **64**, 3033-3041

29. Kehrl, J. H., Rieckmann, P., Kozlow, E., and Fauci, A. S. (1992) Lymphokine production by B cells from normal and HIV-infected individuals. *Ann N Y Acad Sci* **651**, 220-227

30. Kitani, A., Hara, M., Hirose, T., Harigai, M., Suzuki, K., Kawakami, M., Kawaguchi, Y., Hidaka, T., Kawagoe, M., and Nakamura, H. (1992) Autostimulatory effects of IL-6 on excessive B cell differentiation in patients with systemic lupus erythematosus: analysis of IL-6 production and IL-6R expression. *Clin Exp Immunol* **88**, 75-83

31. Burdin, N., Rousset, F., and Banchereau, J. (1997) B-cell-derived IL-10: production and function. *Methods* **11**, 98-111

32. Song, J., Ohkura, T., Sugimoto, M., Mori, Y., Inagi, R., Yamanishi, K., Yoshizaki, K., and Nishimoto, N. (2002) Human interleukin-6 induces human herpesvirus-8 replication in a body cavity-based lymphoma cell line. *J Med Virol* **68**, 404-411

33. Molden, J., Chang, Y., You, Y., Moore, P. S., and Goldsmith, M. A. (1997) A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit. *J Biol Chem* **272**, 19625-19631

34. Aoki, Y., Narazaki, M., Kishimoto, T., and Tosato, G. (2001) Receptor engagement by viral interleukin-6 encoded by Kaposi sarcoma-associated herpesvirus. *Blood* **98**, 3042-3049

35. Chow, D., He, X., Snow, A. L., Rose-John, S., and Garcia, K. C. (2001) Structure of an extracellular gp130 cytokine receptor signaling complex. *Science* **291**, 2150-2155

36. Osborne, J., Moore, P. S., and Chang, Y. (1999) KSHV-encoded viral IL-6 activates multiple human IL-6 signaling pathways. *Hum Immunol* **60**, 921-927

37. Chen, D., Sandford, G., and Nicholas, J. (2009) Intracellular signaling mechanisms and activities of human herpesvirus 8 interleukin-6. *J Virol* **83**, 722-733

38. Hideshima, T., Chauhan, D., Teoh, G., Raje, N., Treon, S. P., Tai, Y. T., Shima, Y., and Anderson, K. C. (2000) Characterization of signaling cascades triggered by human interleukin-6 versus Kaposi's sarcoma-associated herpes virus-encoded viral interleukin 6. *Clin Cancer Res* **6**, 1180-1189

39. Meads, M. (2004) Kaposi's sarcoma-associated herpesvirus-encoded viral interleukin-6 is secreted and modified differently than human interleukin-6 - Evidence for a unique autocrine signaling mechanism. *The Journal of biological chemistry* **279**, 51793-51803

40. Breen, E. C., Gage, J. R., Guo, B., Magpantay, L., Narazaki, M., Kishimoto, T., Miles, S., and Martinez-Maza, O. (2001) Viral interleukin 6 stimulates human peripheral blood B cells that are unresponsive to human interleukin 6. *Cell Immunol* **212**, 118-125

41. Uldrick, T., O'Mahony, D., Aleman, K., Wyvill, K. M., Whitby, D., Bernstein, W., Pittaluga, S., Steinberg, S. M., Little, R. F., and Yarchoan, R. (2009) Dose Zidovudine plus Valganciclovir in the Treatment of Kaposi's Sarcoma-Associated Herpesvirus (KSHV)-Associated Multicentric Castleman's Disease (MCD): A Pilot Study of KSHV Targeted Anti-Tumor Therapy. . in *Kaposi's Sarcoma Associated Herpesvirus and Related Agents 12th International Workshop*, Charleston, S.C.

42. Polizzotto, M. N., Millo, C., Uldrick, T. S., Aleman, K., Whatley, M., Marshall, V., Whitby, D., Steinberg, S. M., Little, R. F., and Yarchoan, R. (2011) [18F]-fluoro-D-deoxyglucose-positron emission tomography findings in Kaposi sarcoma-associated herpesvirus associated multicentric Castleman disease in people with HIV: correlation with clinical, inflammatory and virologic parameters. in *18th Conference on Retroviruses and Opportunistic Infections*, Boston, MA

43. Toedter, G., Hayden, K., Wagner, C., and Brodmerkel, C. (2008) Simultaneous detection of eight analytes in human serum by two commercially available platforms for multiplex cytokine analysis. *Clin Vaccine Immunol* **15**, 42-48

44. Katsume, A., Saito, H., Yamada, Y., Yorozu, K., Ueda, O., Akamatsu, K., Nishimoto, N., Kishimoto, T., Yoshizaki, K., and Ohsugi, Y. (2002) Anti-interleukin 6 (IL-6) receptor antibody suppresses Castleman's disease like symptoms emerged in IL-6 transgenic mice. *Cytokine* **20**, 304-311

45. Beck, J. T., Hsu, S. M., Wijdenes, J., Bataille, R., Klein, B., Vesole, D., Hayden, K., Jagannath, S., and Barlogie, B. (1994) Brief report: alleviation of systemic manifestations of Castleman's disease by monoclonal anti-interleukin-6 antibody. *N Engl J Med* **330**, 602-605.

46. van Rhee, F., Fayad, L., Voorhees, P., Furman, R., Lonial, S., Borghaei, H., Sokol, L., Crawford, J., Cornfeld, M., Qi, M., Qin, X., Herring, J., Casper, C., and Kurzrock, R. (2010) Siltuximab, a novel anti-interleukin-6 monoclonal antibody, for Castleman's disease. *J Clin Oncol* **28**, 3701-3708

47. Mihara, M., Kasutani, K., Okazaki, M., Nakamura, A., Kawai, S., Sugimoto, M., Matsumoto, Y., and Ohsugi, Y. (2005) Tocilizumab inhibits signal transduction mediated by both mIL-6R and sIL-6R, but not by the receptors of other members of IL-6 cytokine family. *Int Immunopharmacol* **5**, 1731-1740

48. Igawa, T., Ishii, S., Tachibana, T., Maeda, A., Higuchi, Y., Shimaoka, S., Moriyama, C., Watanabe, T., Takubo, R., Doi, Y., Wakabayashi, T., Hayasaka, A., Kadono, S., Miyazaki, T., Haraya, K., Sekimori, Y., Kojima, T., Nabuchi, Y., Aso, Y., Kawabe, Y., and Hattori, K. (2010) Antibody recycling by engineered pH-dependent antigen binding improves the duration of antigen neutralization. *Nat Biotechnol* **28**, 1203-1207

49. Hashizume, M., and Mihara, M. (2009) Influence of humanized anti-IL-6R antibody, tocilizumab on the activity of soluble gp130, natural inhibitor of IL-6 signaling. *Rheumatol Int* **29**, 397-401

50. Song, S. N., Tomosugi, N., Kawabata, H., Ishikawa, T., Nishikawa, T., and Yoshizaki, K. (2010) Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. *Blood* **116**, 3627-3634

51. Cannon, J. S., Hamzeh, F., Moore, S., Nicholas, J., and Ambinder, R. F. (1999) Human herpesvirus 8-encoded thymidine kinase and phosphotransferase homologues confer sensitivity to ganciclovir. *J Virol* **73**, 4786-4793.

52. Gustafson, E. A., Schinazi, R. F., and Fingeroth, J. D. (2000) Human herpesvirus 8 open reading frame 21 is a thymidine and thymidylate kinase of narrow substrate specificity that efficiently phosphorylates zidovudine but not ganciclovir. *J Virol* **74**, 684-692

53. Kedes, D. H., and Ganem, D. (1997) Sensitivity of Kaposi's sarcoma-associated herpesvirus replication to antiviral drugs. Implications for potential therapy. *J Clin Invest* **99**, 2082-2086

54. Casper, C., Krantz, E. M., Corey, L., Kuntz, S. R., Wang, J., Selke, S., Hamilton, S., Huang, M. L., and Wald, A. (2008) Valganciclovir for suppression of human herpesvirus-8 replication: a randomized, double-blind, placebo-controlled, crossover trial. *J Infect Dis* **198**, 23-30

55. Casper, C., Nichols, W. G., Huang, M.-L., Corey, L., and Wald, A. (2004) Remission of HHV-8 and HIV-associated multicentric Castleman disease with ganciclovir treatment. *Blood* **103**, 1632-1634

56. Jenner, R. G., Alba, M. M., Boshoff, C., and Kellam, P. (2001) Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. *J Virol* **75**, 891-902

57. Lu, M. (2004) Dissection of the Kaposi's sarcoma-associated herpesvirus gene expression program by using the viral DNA replication inhibitor cidofovir. *Journal of virology* **78**, 13637-13652

58. Berezne, A., Agbalika, F., Oksenhendler, E., Casper, C., Nichols, W. G., Huang, M.-L., Corey, L., and Wald, A. (2004) Failure of cidofovir in HIV-associated multicentric Castleman disease. *Blood* **103**, 4368-4369

59. Bower, M. (2010) How I treat HIV-associated multicentric Castleman's disease. *Blood*

60. Aitken, A. E., and Morgan, E. T. (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos* **35**, 1687-1693

61. Jover, R., Bort, R., Gomez-Lechon, M. J., and Castell, J. V. (2002) Down-regulation of human CYP3A4 by the inflammatory signal interleukin-6: molecular mechanism and transcription factors involved. *FASEB J* **16**, 1799-1801

62. Rivory, L. P., Slaviero, K. A., and Clarke, S. J. (2002) Hepatic cytochrome P450 3A drug metabolism is reduced in cancer patients who have an acute-phase response. *Br J Cancer* **87**, 277-280

63. Mayo, P. R., Skeith, K., Russell, A. S., and Jamali, F. (2000) Decreased dromotropic response to verapamil despite pronounced increased drug concentration in rheumatoid arthritis. *Br J Clin Pharmacol* **50**, 605-613

64. Fournier, T., Medjoubi, N. N., and Porquet, D. (2000) Alpha-1-acid glycoprotein. *Biochim Biophys Acta* **1482**, 157-171

65. Schmitt, C., Kuhn, B., Zhang, X., Kivitz, A. J., and Grange, S. (2011) Disease-Drug-Drug Interaction Involving Tocilizumab and Simvastatin in Patients With Rheumatoid Arthritis. *Clin Pharmacol Ther*

66. Frey, N., Grange, S., and Woodworth, T. (2010) Population pharmacokinetic analysis of tocilizumab in patients with rheumatoid arthritis. *J Clin Pharmacol* **50**, 754-766

67. Cheson, B. D., Horning, S. J., Coiffier, B., Shipp, M. A., Fisher, R. I., Connors, J. M., Lister, T. A., Vose, J., Grillo-Lopez, A., Hagenbeek, A., Cabanillas, F., Klippensten, D., Hiddemann, W., Castellino, R., Harris, N. L., Armitage, J. O., Carter, W., Hoppe, R., and Canellos, G. P. (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol* **17**, 1244

68. Cheson, B. D., Pfistner, B., Juweid, M. E., Gascoyne, R. D., Specht, L., Horning, S. J., Coiffier, B., Fisher, R. I., Hagenbeek, A., Zucca, E., Rosen, S. T., Stroobants, S., Lister, T. A., Hoppe, R. T., Dreyling, M., Tobinai, K., Vose, J. M., Connors, J. M., Federico, M., and Diehl, V. (2007) Revised response criteria for malignant lymphoma. *J Clin Oncol* **25**, 579-586

69. Yarchoan, R., and Broder, S. (1987) Progress in the Development of Antiviral Therapy for HTLV-III-Associated Diseases. in *Important Advances in Oncology 1987* (Vincent T. DeVita, J., Hellman, S., and Rosenberg, S. A. eds.), J.B. Lippincott Company, Philadelphia. pp 293-311

12. APPENDICES

12.1. APPENDIX 1: KSHV-MCD CLINICAL BENEFIT RESPONSE CRITERIA

KSHV-MCD Clinical Benefit Response Criteria has been developed as a more streamlined tool for assessment of patients receiving therapy for KSHV-MCD, and does not depend on radiographic findings. As such, it allows for same-day assessment of clinical benefit utilizing common symptoms and clinical laboratories. To evaluate clinical benefit in patients with KSHV-MCD receiving therapy, clinical and laboratory responses are assessed in aggregate, and compared to baseline. This criterion allows for assessment of patients who may be deriving meaningful clinical benefit from therapy, but have not necessarily achieved an overall complete response by the original NCI KSHV-MCD response criteria.

Evaluation of KSHV-MCD is complicated by the heterogeneous and non-specific nature of many symptoms and signs of KSHV-MCD and the common intercurrence of other pathologies in this group. For this reason, assessment of responses other than complete response is made on the basis of eight *indicator abnormalities* (four symptom groups and four laboratory parameters) that are most closely associated with disease activity:

Indicator Abnormalities Assessed in Responses	
Symptoms	*Laboratory Abnormalities*
Fever (includes chills and rigors)	Elevated C-reactive protein
Fatigue (includes lethargy)	Thrombocytopenia
Gastrointestinal (includes nausea and anorexia)	Anemia
Respiratory (includes airway hyperreactivity and cough)	Hypoalbuminemia

For the purposes of response assessment, clinical symptoms attributed to MCD will be assigned an NCI-CTCAE grade equivalent, with response assessment based on changes in grade severity or symptom resolution.

Increases in hemoglobin in patients who have received a transfusion do not count towards PR or CR for 3 weeks, and increases in albumin or platelet count in patients who have received a transfusion do not count towards PR or CR for 7 days.

Complete Response (CR):

Full resolution of all clinical symptoms and laboratory abnormalities (whether or not these are indicator abnormalities) probably or definitely attributable to MCD, lasting at least 3 weeks.

Partial Response (PR):

PR is assessed based on the eight indicator abnormalities above. At least 50% of the abnormalities probably or definitely attributed to KSHV-MCD must improve by the minimum amounts specified below to attain PR.

Only abnormalities present in a specific patient at baseline may count toward the achievement of a PR (e.g. if six of indicator abnormalities are present at baseline, at least three must meet the specified criteria to be considered a PR).

Improvement in symptoms require at least 1 CTCAE grade equivalent improvement. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KSHV-MCD must improve by at least 1 CTCAE grade equivalent to consider the group as a whole to be improved.

Improvement in for each laboratory parameters requires either normalization of the lab value or else the following:

- C-reactive protein reduction to ≤50% of baseline
- Hemoglobin increment 2g/dL not explained by transfusion
- Platelet increment ≥50 K/uL not explained by transfusion
- Albumin increment ≥1g/dL not explained by transfusion

To be considered a partial response, there may be no new indicator abnormalities probably or definitely attributed to KSHV-MCD; no indicator symptom may worsen by ≥1 CTCAE grade equivalent; and no indictor laboratory abnormality may worsen by the amount given in the criteria for progressive disease.

Stable disease (SD): no change in signs and symptoms of KSHV-MCD that meet criteria for any of CR, PR or PD.

Progressive disease (PD):

PD is assessed based on the eight indicator abnormalities above. At least two indicator abnormalities must deteriorate by the minimum amounts specified below to constitute PD. The development of new indicator abnormalities not present in a specific patient at baseline is incorporated in the assessment of PD

Deterioration in signs and symptoms require at least 1 CTCAE grade equivalent increase in severity. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KSHV-MCD must increase in severity by at least 1 CTCAE grade equivalent to consider the group as a whole to have deteriorated.

Deterioration for each laboratory parameter requires an abnormal laboratory value meeting the following criteria:

- C-reactive protein increase by ≥50% of baseline (or the upper limit of normal, whichever is greater)
- Hemoglobin decrement 2g/dL not otherwise explained
- Platelet decrement ≥25 K/uL not otherwise explained
- Albumin decrement ≥0.5g/dL

12.2. APPENDIX 2: KSHV-ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE RESPONSE CRITERIA (EVALUATED IN 04-C-0275)

Response Category	Response and Criteria
Clinical Response	*Complete Response (CR)*

	Full resolution of all signs and symptoms attributable to MCD, lasting 1 cycle (3-4 weeks depending on regimen). Requires normalization of involved nodal areas on physical exam. *Symptom Free Disease (SFD)* Full resolution of all symptoms attributable to MCD. *Partial Response (PR)* Improvement in at least 50% of signs and symptoms by at least 1 grade (NCI-CTCAE v3), with no increased MCD related increases, lasting 1 cycle (3-4 weeks depending on regimen) *Stable Disease (SD)* No change in signs and symptoms of MCD meeting criteria for CR, PR or PD. *Progressive Disease (PD)* Worsening of 2 or more symptoms by at least 1 Grade.
Biochemical Response	*Complete Response (CR)* Normalization of abnormalities attributed to MCD in the following labs: hemoglobin, platelets, albumin, sodium and C-reactive protein (CRP), lasting 1 cycle (3-4 weeks depending on regimen). *Partial Response (PR)* At least 50% improvement in <u>all</u> labs abnormalities attributable to MCD, lasting 1 cycle (3-4 weeks depending on regimen). *Stable Disease (SD)* No change in biochemical parameters that meet criteria for CR, PR or PD *Progressive Disease (PD)* ≥ 25% and 1 Grade worsening of at least 2 biochemical parameters attributable to MCD <u>OR</u> clear deterioration in one parameter with negative impact on physiologic or health status
Radiographic Response	*Complete Response (CR)* Normalization of all lymph nodes to <1.5 cm in greatest transverse dimension, with decrease to < 1 cm of lymph nodes that measure 1.1-1.5 cm at baseline, or 75% decrease in sum products of the diameters (SPD) of measured lymph nodes. Spleen < 12 cm greatest dimension, no pleural effusions. *Complete Response, unconfirmed (CRu)* Residual lymph node mass >1.5 cm or spleen > 12 cm that has decrease by ≥75% and does not change over one year. *Partial Response (PR)* For lymph nodes, ≥ 50% decrease in SPD of 6 dominant nodes; for spleen ≥ 50% decrease in longest transverse dimension. *Stable Disease (SD)* Not meeting criteria for CR, CRu, PR or PD.

	Progressive Disease (PD) For lymph nodes ≥ 25% increase in the SPD, for spleen increase ≥ 25% in longest dimension.
Overall Response	*Complete Response (CR)* CR in all categories. *Partial Response (PR)* PR or better in all categories. *Stable Disease (SD)* SD or better in all categories. *Progressive Disease (PD)* Progression in any one category.

Based on signs and symptoms probably or definitely attributed to KSHV-MCD.

Grade: Based on signs, symptoms and laboratory values Probably or Definitively Attributed to MCD

Grade 1 (meets all three criteria):

- ECOG 0-1
- All constitutional symptoms ≤1
    - Fatigue
    - Fever
    - Rigors/chills
    - Diaphoresis
    - Weight gain/edema
    - Weight loss/anorexia
- All Laboratory Abnormalities ≤ Grade 2, maximum 1 grade 3.
    - Hgb
    - Platelets
    - Leukopenia
    - Albumin
    - Na
    - CRP < 8 mg/L

Grade 2: Not grade 1 or Grade 3

Grade 3 (meets all 3 criteria):

- ECOG 3-4
- 2 or more symptoms Grade 4 by CTCAE
    - Fatigue
    - Fever
    - Rigors/chills
    - Diaphoresis
    - Weight gain/edema
    - Weight loss/anorexia
- Any 2 or more laboratory abnormalities (above) ≥ Grade 3, including CRP >30 mg/L

OR

- Tumor or Disease threatens a vital organ

12.3. APPENDIX 3: MCD RESPONSE ASSESSMENT TOOLS

HAMB MCD AND KICS RESPONSE SUMMARY

INITIALS		STUDY		KEEP IN RESEARCH RECORD ONLY

SYMPTOMS AND LABS	DATE (CYCLE/DAY)_____ (___/___)				DATE (CYCLE/DAY)_____ (___/___)				DATE (CYCLE/DAY)_____ (___/___)			
	VALUE*	GRADE†	RESEARCH	DISEASE	VALUE*	GRADE†	RESEARCH	DISEASE	VALUE*	GRADE†	RESEARCH	DISEASE

RESPONSE THRESHOLD	PR	PD
CRP		
HEMOGLOBIN		
PLATELETS		
ALBUMIN		

CLINICAL	
BIOCHEMICAL	
UNIFIED RESPONSE	
RADIOGRAPHIC§	
CONFIRMED BY	

CLINICAL	
BIOCHEMICAL	
UNIFIED RESPONSE	
RADIOGRAPHIC§	
CONFIRMED BY	

*VALUES FOR LABORATORY INDICES ONLY. †CTCAE GRADE. §NOT FOR KICS PATIENTS. ATTRIBUTION: 1. UNRELATED 2. UNLIKELY 3. POSSIBLE 4. PROBABLE 5. DEFINITE

MCD Clinical, Biochemical and Radiographic Responses By Cycle

Patient Name_____**MRN**_____

Regimen _____

			Last Day of Cycle							Day 1
Date	**Drugs**	**Cycle**	**Clinical Response**	**I, S, M, W**	**Biochemical Response**	**I, S, M, W**	**Radiographic Response LN / Spleen**	**I, S, M, W**	**Overall**	**Grade**
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
							/			
Overall Responses										
							/			

CR = Complete Response, SFD = symptom free disease, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease.
I = Improved, S= Stable, M= Mixed Response, W= Worse: Used to note cycle-to-cycle direction of response for patients with improvement or worsening that is categorized as SD or PR.
Drugs: Toc = Tocilizumab, A/G= AZT + (val)ganciclovir, R-D = Rituximab + Doxil, I = interferon α (list dose), EPOCH-RR, B-A/G: bortezomib + A/G, O= other combination (list)

12.4. APPENDIX 4: MODIFIED ACTG KAPOSI SARCOMA RESPONSE WORKSHEETS

HAMB KAPOSI SARCOMA MEASUREMENT AND RESPONSE SUMMARY

INITIALS		DATE		STUDY		CYCLE		PHOTOS	
BASELINE TIS		T:	I:	S:		REASON T1:			

KEEP IN RESEARCH RECORD ONLY

LESION	DESCRIPTION	FLAT OR NODULAR	DIMENSIONS	PRODUCT
ONE				
TWO				
THREE				
FOUR				
FIVE				
			TOTAL PRODUCT	

TOTAL LESIONS	OVER 50_____ UNDER 50*_____	*FOR PATIENTS WITH UNDER 50 LESIONS, WRITE 'TOTAL BODY' FOR AREA AND USE LEFT COLUMN

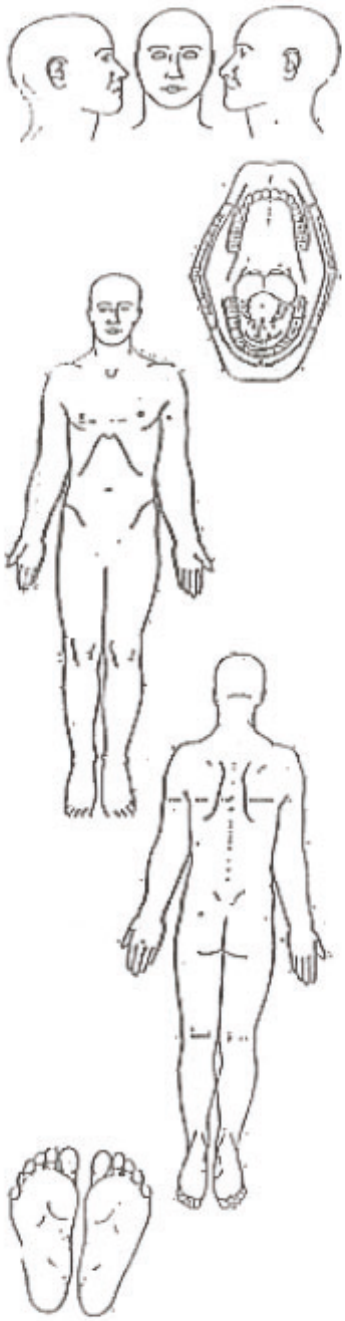
AREA			AREA			
NUMBER FLAT			NUMBER FLAT			
NUMBER NODULAR			NUMBER NODULAR			
TOTALS	ALL		NODULAR		FLAT	

ORAL LESIONS	PRESENT		NONE		NO EVAL.	
DESCRIPTION IF PRESENT						
VISCERAL LESIONS	PRESENT		NONE		NO EVAL.	
DESCRIPTION IF PRESENT						

RESPONSE THRESHOLDS FROM: _____				RESPONSE FROM BASELINE	
PARTIAL RESPONSE		PROGRESSIVE DISEASE		RESPONSE BASED ON	
TOTAL		TOTAL		RESPONSE FROM _____	
NODULAR		NODULAR		RESPONSE BASED ON	
PRODUCT		PRODUCT		RESPONSE CONFIRMED BY	

RECORDER	SIGNATURE

HAMB KAPOSI SARCOMA MEASUREMENT AND RESPONSE SUMMARY

REGIONS	LESION COUNT
	

TUMOR EDEMA					
LEFT			RIGHT		
LEVEL	DIST.	DIAM.	LEVEL	DIST.	DIAM.
ANKLE	0cm		ANKLE	0cm	
KNEE			KNEE		
THIGH			THIGH		
PELVIS			PELVIS		

12.5. APPENDIX 5: PERFORMANCE STATUS SCALES

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.