Supplementary Table 1

KSHV-MCD Clinical Benefit Response Criteria

Response Criteria

For the purposes of response assessment, clinical symptoms attributed to MCD were assigned an NCI-CTCAE grade equivalent, with response assessment based on changes in grade severity or symptom resolution. Increases in hemoglobin in patients who received a transfusion did not count towards PR or CR for 3 weeks and increases in albumin or platelet count in patients who have received a transfusion did 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)

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
• 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

Supplementary Table 2: Laboratory, cytokine and immunologic markers at baseline and median paired changes at C1D2 (48 hours following first tocilizumab infusion), Cycle 2 (2 weeks after first infusion) and end of therapy.

	Baseline (IQR)	**Median paired difference – Baseline to C1D2 (IQR)**	P	**Median paired difference – Baseline to C2 (IQR)**	P	**Median paired difference – Baseline to end-of study (IQR)**	P
Response Criteria Laboratory values							
Hemoglobin, g/dL	10.5 (9.4-12.2)	0 (-0.5-0.5)	1.00	0.9 (0.6-2.0)	0.19	0.9 (-1.1 – 2.5)	0.38
Platelets K/µL	218 (157.8-340)	+32 (-24-53)	0.16	-39.5 (-118 – 11.5)	0.20	-47.0 (-119.5-16.5)	0.33
Albumin g/dL	3.2 (2.8-3.4)	0.3 (-0.1-0.3)	0.28	+0.6 (0.4 – 1.1)	0.08	0.7 (0.15-1.25)	0.06
CRP mg/L	23 (12.8-98.3)	-10.3 (-49.2- -5.4)	0.02	-16.5 (-102.7- -10.2)	0.08	-19.9 (-93.2-57.7)	0.64
Cytokines (pg/ml)							
sIL6-R	32,999.5 (29526-42017)	+77,639 (67694-87577)	0.02	+192,991 (182944- 210799)	0.008	+204305 (198054-224674	0.008
hIL-6	3.3 (1.8-6.9)	+28.6 (23.4-79.2)	0.02	+23.4 (13.5- 73.7)	0.008	+48.3 (18.1 – 126.2)	0.02
IFN-γ	26.2 (9.8-81.7)	+1.8 (-1.7-3.5)	0.47	+4.5 (-3.9-17.2)	0.46	+15.5 (-38.9- 274.1)	0.31
IL-10	16.0 (7.2-133.5)	-2.9 (-8.0 – 7.8)	0.69	-9.3 (-174.8 – 7.3)	0.31	-14.8 (-154.7 – 528.3)	1.00
IL-1B	0.7 (0.6-0.8)	0 (-0.2-0.03)	1.00	-0.04 (-0.1 – 0.03)	0.58	-0.1 (-0.2-0.2)	0.59
Immunologic markers							
KSHV log_{10} copies/10^6 PBMCs	4.1 (3.6-4.3)	-	-	-0.7 (-1.8 – 0.5)	0.2	0.05 (-1.4 – 1.7)	1.00
CD4 cells/µL	232.5 (121.5-492.5)	+30.5 (5.3-104.5)	0.04	+21 (-27-234.3)	0.33	+8.5 (6.3-38.8)	0.16
CD19 cells/µL	140.5 (50.5-249)	-24 (-59.3- -11.3)	0.04	-27 (-57.8 – 6)	0.32	-44.5 (-107.5 – 3.3)	0.12
K free light chains mg/dL	8.7 (4.7 – 18.4)	-3.3 (-7.4-0.5)	0.24	-3.3 (-12.1 -2.2)	0.21	-2.0 (-7.2-0.2)	0.26
L free light chains mg/dL	6.2 (5.4 – 7.1)	-1.2 (-2.9 – 0.3)	0.61	-2.6 (-4.1- -0.2)	0.26	-0.5 (-1.9 – 0.8)	0.58

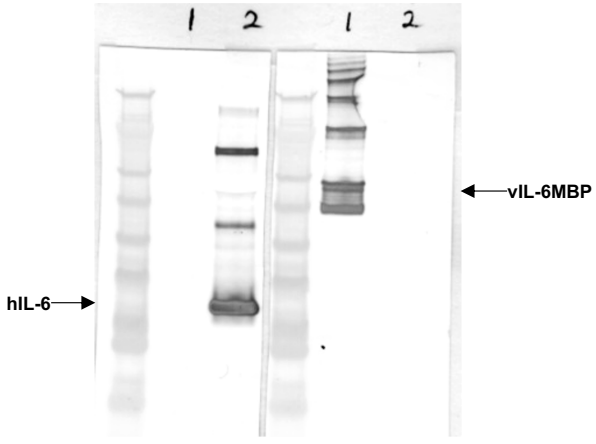
Supplementary Table 3: Selected adverse event that were possibly, probably or definitely attributed to research (tocilizumab with or without AZT/VGC) over 39 cycles in 8 patients.						
Toxicity	**Grade 1**		**Grade 2**		**Grade 3**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Tocilizumab monotherapy – 8 patients, 29 cycles						
Thrombocytopenia						
Events	2	18	2	18		
Patients	2	25	2	25		
Neutropenia						
Events			1	9		
Patients			1	12		
Elevated liver enzymes						
Events	2	18				
Patients	1	12				
Cholesterol elevated						
Events	2	18				
Patients	2	25				
Diarrhea						
Events	1	9				
Patients	1	12				
Pruritis						
Events			1	9		
Patients			1	12		
Tocilizumab in combination with AZT/VGC – 3 patients, 10 cycles						
White cell count decreased						
Events			2	15		
Patients			2	67		
Neutropenia						
Events			2	15	2	15
Patients			2	67	2	67
Thrombocytopenia						
Events			2	15		
Patients			1	33		
Leukopenia						
Events			2	15		
Patients			2	67		
Creatinine increased						
Events	1	8				
Patients	1	33				
Nausea						
Events	2	15				
Patients	2	67				

Supplementary Figure 1. Assessment of the binding of siltuximab (CNTO 328) to KSHV-encoded vIL-6 by Western blot and enzyme-linked immunosorbent assay (ELISA)

Western blot methods: A purified fusion protein of maltose binding protein (42.7Kd) and amino acids 22 to 204 of viral IL-6, 21.6Kd (vIL-6MBP) and human recombinant IL-6 (hIL-6) (R&D System. 20.3kDa) proteins were solubilized in SDS sample buffer (Invitrogen, San Diego, CA), heated at 94^0C for 10 minutes, cooled down in ice for 2 minutes, and then electrophoresed on precast 4-12% Tris-Bis NuPAGE gel (Invitrogen) (References 1-2). Proteins were then transferred onto nitrocellulose membranes using iBlot (Invitrogen). Membranes were blocked in 5% w/v nonfat dry milk in Tris buffered Saline pH7.4 containing 0.05% Tween 20 (1XTBST) solution at 4^0C overnight. After washing three times with 1XTBST membranes were incubated with either mouse anti-hIL-6 antibody (CNTO 328, lot 761801, kindly provided by Johnson and Johnson) or mouse anti-vIL-6 antibody at 0.5 µg/ml in blocking buffer for two hours at room temperature. Membranes were incubated with a goat anti-mouse secondary antibody conjugated to alkaline phosphatase (Promega 1:2000) for one hour following the three times washing with 1XTBST, and bands were visualized with stabilized Western Blue substrate (Promega).

ELISA methods: Wells of a polystyrene 96-well plate (Immulon, Thermo Scientific, Waltham, MA) were coated with recombinant hIL-6 protein (R&D) or vIL-6-MBP at 0.2 µg/ml in phosphate buffered saline (PBS) pH7.4 (60 µl/ml) or, as a negative control, PBS alone overnight at 4 ^{0}C. After removing the coating protein or PBS, wells were blocked with superblock buffer (Pierce) 300ul/well. Serially diluted human IL-6 antibody (CNTO 328) or rabbit vIL-6 polyclonal antibody (references 1,2) from 0.4 µg/ml to 0.39 ng/ml)in PBS with 0.05% Tween 20 (PBST) and 0.5% bovine serum albumin (PBST/BSA) were added to the wells (200 µl/well) and incubated for two hours at room temperature. After four times washing with PBST, affinity purified goat anti-human (Promega 1:2500 dilution) or anti-rabbit (Bio-Rad 1:5000 dilution) antibodies conjugated to horseradish peroxidase diluted with PBST/BSA solution were added and incubated for two hours at room temperature. Following an addition washing, SureBlue TMB microwell peroxidase substrate (KPL Inc, Gaithersburg, MD) was added to the wells, incubate for 10 minutes, followed by stop solution (1N HCl) and plate was read at 450 nm with correction at 630 nm.

Western blot results showing the lack of binding of CNTO 328 to vIL-6MBP



Western blot results: Purified vIL-6-MBP at 20 ng/lane) (lane 1) and hIL-6 protein at 100 ng/lane (lane 2) were used in Western Blots. The two transferred membranes were probed with anti-hIL-6

antibodies (left blot) and anti-vIL-6 antibody (right blot) under same conditions as described. For each blot, the left-hand lane shows size markers. hIL-6 antibody (CNTO 328, lot 761801) recognized recombinant human IL-6 protein (left blot, lane 2) but did not detect vIL-6-MBP (left blot, lane 1). Mouse monoclonal antibody to vIL-6 recognized the vIL-6 MBP at 20 ng/lane (right blot, lane 1), but failed to react with hIL-6 recombinant protein (right blot, lane 2).

ELISA assay results: Two groups of three rows of wells in a 96-well ELISA plate were first coated with recombinant hIL-6, vIL-6 MBP, or PBS control followed by incubation with serially diluted anti hIL-6 (CNTO 328) or anti vIL-6 antibody in concentrations ranging from 0.4 µg/ml to 0.39 ng/ml. Monoclonal antibody to human IL-6 (CNTO 328) could detect recombinant hIL-6 protein at a concentration as low as 0.78 ng/ml of antibody, but failed to detect vIL-6 MBP even at the highest concentration of 0.4 µg/ml of antibody. Purified rabbit anti vIL-6-MBP antibody, on the other hand, could detect viral IL-6 MBP at a concentration as low at 0.39 ng/ml of antibody, but did not show any signal above background against failed hIL-6 protein at 0.4 µg/ml.

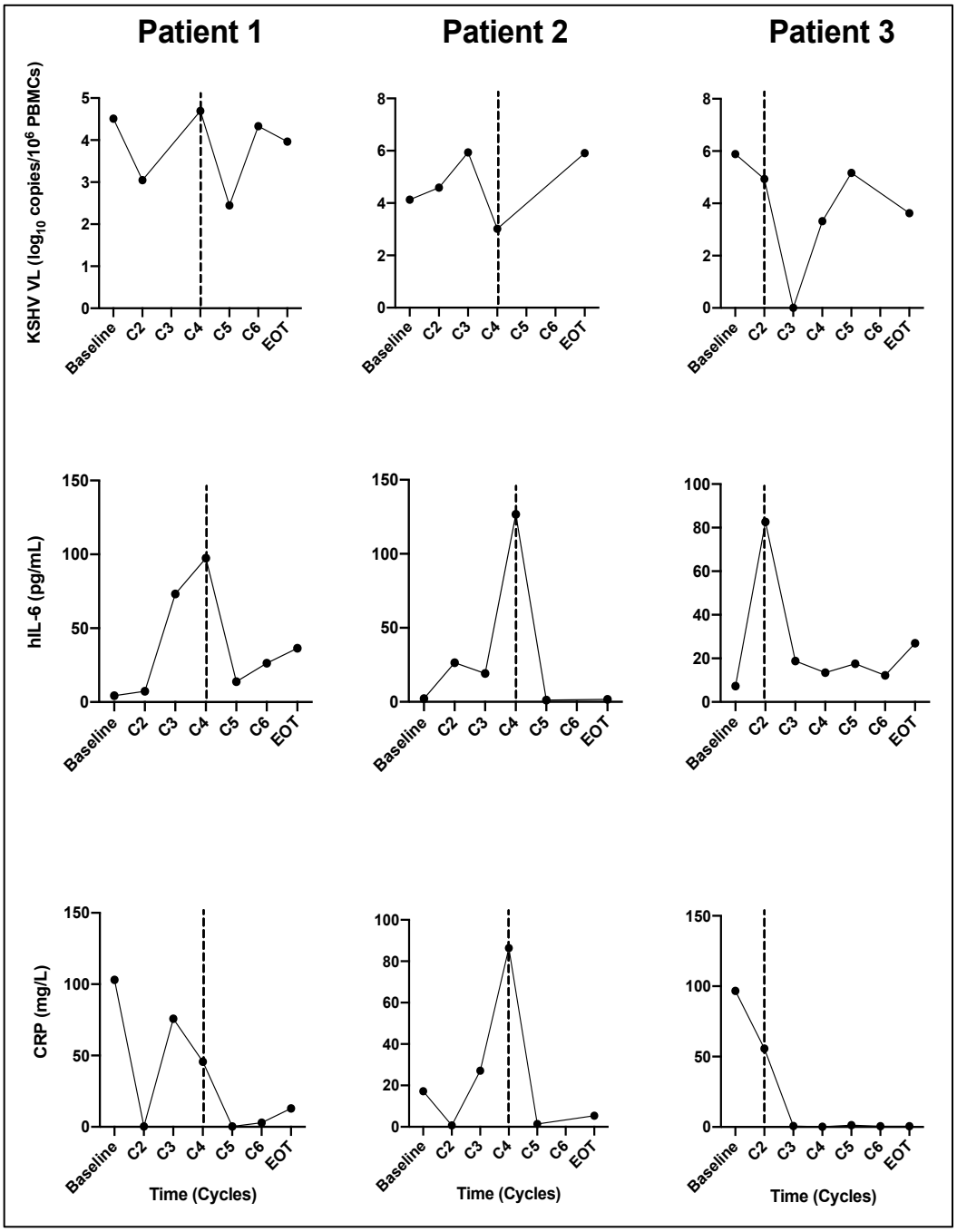
We concluded that CNTO 328 anti-hIL-6 did not bind to vIL-6-MBP fusion protein. It is worth noting that vIL-MBP contains only amino acids 22- 204 of vIL-6. However, it is extremely unlikely that CNTO 328 would bind to this region of vIL-6, as there is almost no homology between hIL-6 and vIL-6 in that region, and these amino acids represent the N-terminal signal peptide of vIL-6.

References:

1. Aoki Y, Jaffe ES, Chang Y, et al. Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. Blood. 1999; 93: 4034-4043.

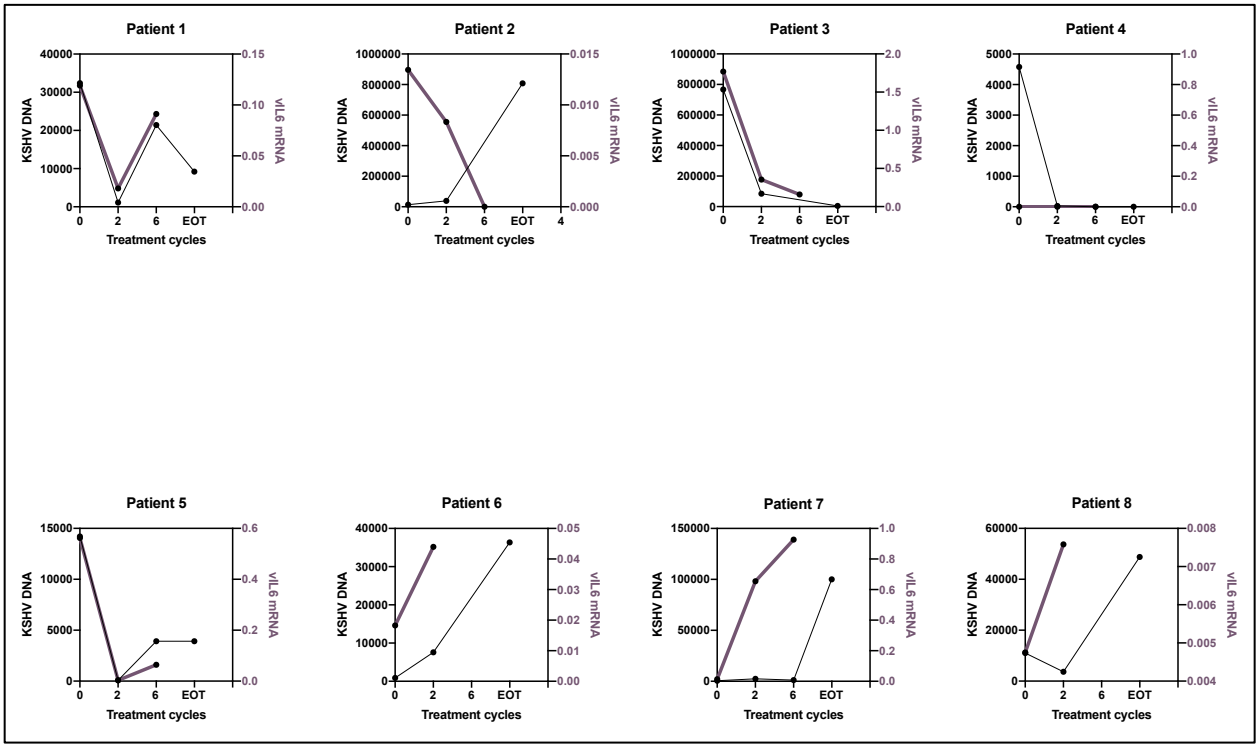
2. Jones KD, Aoki Y, Chang Y, Moore PS, Yarchoan R, Tosato G. Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells. Blood. 1999; 94: 2871-2879.

Supplementary Figure 2



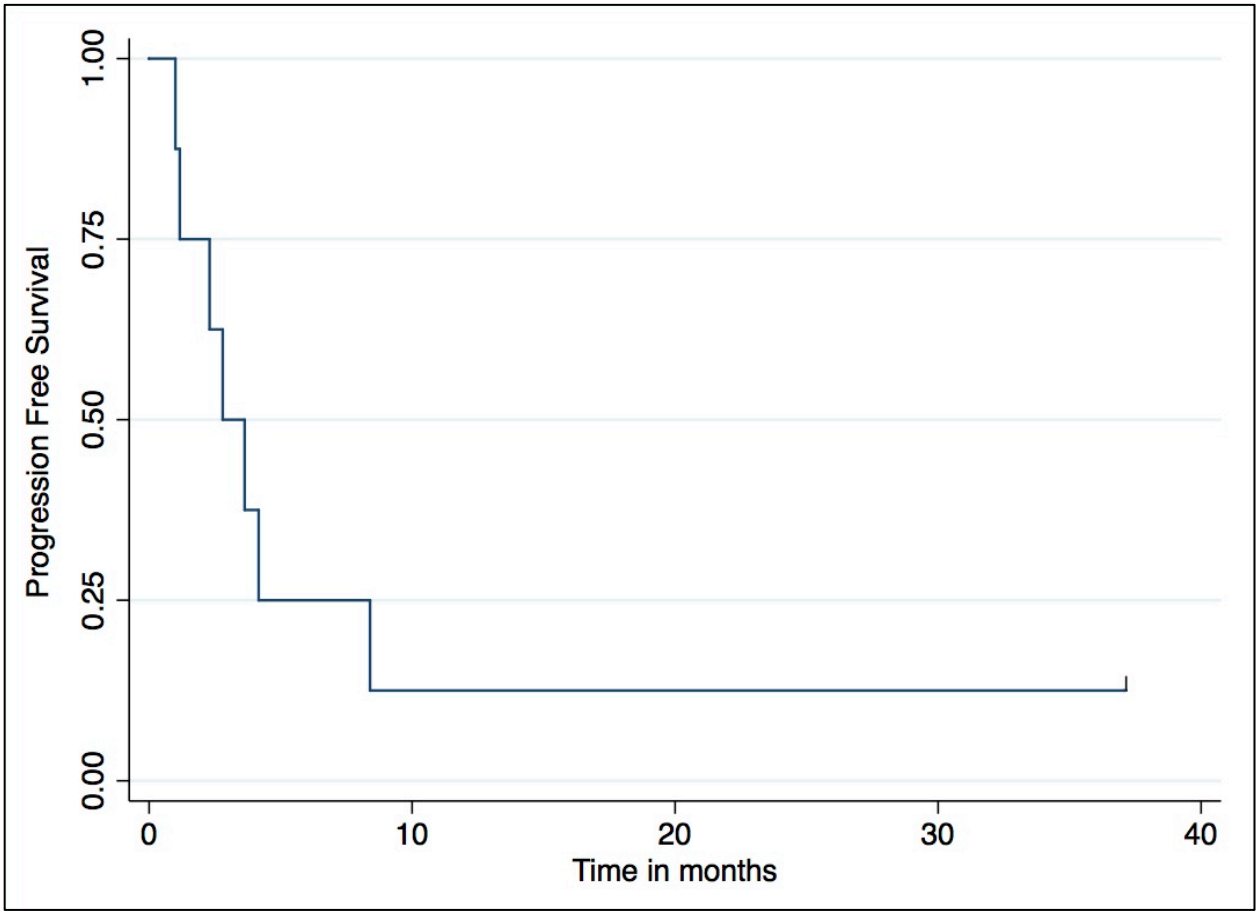
Changes in KSHV VL, CRP and IL6 among patients administered tocilizumab, AZT and VGC. Dotted lines represent the addition of AZT/VGC to tocilizumab.

Supplementary Figure 3



KSHV-DNA levels within PBMCs (copies/million cell equivalents) (heavy lines) and viral IL6 mRNA levels (light lines) at baseline, cycle 2, cycle 6 and end of treatment visits among all patients treated on study.

Supplementary Figure 4



Progression-free survival for all patients treated on study with tocilizumab with or without AZT and VGC.